Supplement to the
"Proceedings of the Indian Academy of Sciences"

PROCEEDINGS

OF THE

INDIAN ACADEMY OF SCIENCES

VOL. XLVI

SECTION B

BANGALORE CITY
PRINTED AT THE BANGALORE PRESS, MYSORE ROAD
1958

Φ
73
.I36
no. B
v. 46

CONTENTS

SECTION B—VOL. XLVI

No. 1—July 1957

PAGE

Oceanographic Studies in the Bay of Bengal . . . . . E. C. LaFond 1

Ionic Changes in Unstriated Muscle Immersed in Sodium-Free Solutions . . . . . Inderjit Singh and A. K. Acharya 47

Electron Microscopy and Algology . . . . . T. V. Desikachary 54

Translocation of Griseofulvin in Rice Seedlings (*Oryza sativa* L.) . . . . . N. S. Subba-Rao 65

Embryological Studies in Compositæ. III. *Gerbera jamesonii* Bolus . . . . . H. Maheswari Devi 68

No. 2—August 1957

***Chilo tumidicostalis* Hampson—A Serious Stem Borer Pest of Sugarcane in Bihar** . . . . . K. L. Khanna, L. N. Nigam and V. D. Puri 75

Studies on the Anatomy of the Sugarcane Moth Borers. III. Comparative Study of the Head and Its Appendages . . . . . V. D. Puri 96

Phosphorus Cycle in the Sea with Particular Reference to Tropical Inshore Waters . . . . . R. Jayaraman and G. Seshappa 110

A New Report of a Rust [*Puccinia kuehnii* (Krueg) Butler] on *Erianthus munja* . . . . . S. L. Sharma 126

Influence of *Fusarium* Culture Filtrates on Respiratory Changes in Cotton . . . . . M. Lakshmanan and C. S. Venkata Ram 131

The Metabolism of Serine in Pyridoxine Deficiency . . . . . G. B. Nadkarni and A. Sreenivasan 138

No. 3—September 1957

PAGE

Notes on Some Fungi from South India—VI . . . T. S. Ramakrishnan 149

The Relation between Loss in Viability and Seed-Borne Micro-Flora in Rice . . . . . S. Y. Padmanabhan 155

Studies on the Egg Masses and Larval Development of Some Prosobranchs from the Gulf of Mannar and the Palk Bay . . . A. V. Natarajan 170

No. 4—October 1957

The Metabolism of Serine in Biotin Deficiency . . . . . G. B. Nadkarni and A. Sreenivasan 229

The Metabolism of Serine in Folic Acid Deficiency . . . . . G. B. Nadkarni and A. Sreenivasan 236

Hymenia recurvalis **F. and Its Parasite Complex** . . . . . E. S. Narayanan, B. R. Subba Rao and M. Ramachandra Rao 241

Contribution to Our Knowledge of the Physiological Anatomy of Some Indian Hydrophytes. VI. The Stem of *Hygrophila quadrivalvis* Nees . . . . . M. V. Mirashi 247

Some Observations on the Reproduction of the Milkfish *Chanos chanos* (Forskål) . . . . . P. R. S. Tampi 254

Assimilation of Disaccharides by Some Fungi Causing "Leaf Spot" Diseases . . . . . R. N. Tandon and K. S. Bilgrami 274

Excitation, Inhibition, Contraction, Action Potentials and Conduction in Unstriated Muscle in the Absence of any Electrolyte in the External Medium . . . . . Inderjit Singh and A. K. Acharya 285

No. 5—November 1957

Thermal Field and Oceanic Circulation along the East Coast of India . . . . . A. A. Ramasastry and C. Balaramamurty 293

Hyphomycetes—IV . . . . . C. V. Subramanian 324

***Taenioma* J. Ag. from India** . T. V. Desikachary and M. S. Balakrishnan 336

No. 6—December 1957

PAGE

The Development of the Chondrocranium in *Trichopodus trichopterus* (Pall.) . . . . . V. B. Marathe and D. V. Bal 347

Some New Species of Indian Hymenoptera . . . . . B. R. Subba Rao 376

Studies in Sampling Technique. VI. Rind Hardness in Sugarcane . . . . . S. L. Sharma and Rajeshwar Prasad 391

OCEANOGRAPHIC STUDIES IN THE BAY OF BENGAL

By E. C. LaFond*

Received January 4, 1957

(Communicated by Sir C. V. Raman)

TABLE OF CONTENTS

	Page
Introduction	1
Oceanographic Programs	2
Studies in Marine Geology	
Sea Floor Topography	5
Sea Floor Sediments	9
Beach Erosion	12
Studies in Marine Zoology	
Sea Surface Features and Water Transparency	19
Plant Nutrients and other Chemicals ..	21
Marine Organisms	25
Studies in Physical Oceanography	
Water Temperature and Density	28
Water Circulations	36
Conclusions	42
Acknowledgments	43

Introduction

Indian Ocean.—The Indian Ocean remains one of the unexplored frontiers of our earth. It encompasses nearly 3,000 miles of coast-line and stretches hundreds of miles to the south, east, and west. It has an area 23 times as great as that of India itself and is the only major ocean named after a country. It is only fitting, therefore, that the study of this vast

* The author was Visiting Professor of Oceanography at Andhra University during 1952–53 and 1955–56. His services were made available through the U.S. Educational Exchange Program (Fulbright). He is currently Head of the Physical Oceanography Section of the U.S. Navy Electronics Laboratory, San Diego, California.

body of water has been taken up as a major subject here in India. The Andhra University, a pioneer in many fields, has introduced the scientific study of the north-eastern part of the Indian Ocean—the Bay of Bengal—into its curriculum, and some of the progress in this field is here reported

Bay of Bengal.—The Bay of Bengal has many fascinating and unique features. The offshore area lying between 8 and 22 degrees north is clear blue tropical water. The nearshore waters, on the other hand, undergo seasonal cycles of color, varying from deep blue to the greenish hue of plankton and the brown of coastal drainage.

The wide changes in the physical and chemical properities of the water and its circulation are the result of unique meteorological conditions associated with the monsoons. These not only completely change the current direction with the season, but monsoons also produce the largest rainfall and dilution in the world.

From a geological standpoint, the Bay of Bengal is famous for its unprecedented drainage and the associated deposition of sediments. The submarine canyon, "Swatch of No Ground," and the littoral sand drift along the east coast beaches are world renowned. Oceanography of the Bay of Bengal is more complex and intriguing than that of any other similar body of water and many fascinating problems await only investigation.

OCEANOGRAPHIC PROGRAMS

Previous expeditions.—In the past, several expeditions have studied oceanographic features of parts of the Indian Ocean—namely, the research vessels Challenger, Planet, Dana, Valdivia, Mahabiss, Discovery, Snellius, Gauss, Novarra, Siboga, etc. None have been sustaining studies, that is, repeated or systematically planned for the entire ocean. The main oceanographic work in the Bay of Bengal was done from the Investigator, operated by the British and running from Ceylon to the Andaman Islands, but sometimes veering to Madras or Calcutta. These are nearly all surface observations and were reported in the *Memoirs of the Asiatic Society of Bengal* by Col. Sewell a quarter of a century ago. Within the last ten years two round-the-world deep sea expeditions, the Danish Galathea and the Swedish Albatross, have traversed the Bay of Bengal. The Albatross passed through only the southern part, but the Galathea took soundings up to Calcutta. No deep oceanographic stations have been occupied in the Bay and it still remains to be thoroughly investigated.

Present studies.—The Central Government is currently sponsoring some oceanographic work. The new INS Investigator has been employed for

oceanographic measurements, along with hydrographic surveys. The Survey of India has a long record of tides, and publishes a forecast for Indian Ports. The Meteorological Department has been collecting meteorological and current data from merchant vessels plying the Indian Ocean. Also, the Department of Fisheries Research has initiated measurements of temperatures, surface currents, and chemical properties of the water in several near-shore areas, together with studies of the biological populations, especially commercial fish. Another recent agency is the Indian Navy Physical Laboratory which has initiated several oceanographic studies along the west coast. However, the group most concerned with the development of oceanographic research in the Bay of Bengal is the Andhra University at Waltair.

Andhra University.—Most of the credit for the oceanographic program at Andhra University must be given to the Vice-Chancellor, Professor V. S. Krishna, who first realized the desirability for oceanographic instruction in India and took the necessary steps to provide facilities and to obtain assistance in getting the program started. And he has made sure that once initiated, the work would continue and expand.

The academic teaching of oceanography had been carried on in the Geology, Zoology, and Geophysics Departments for a number of years. Offshore oceanographic research, however, dates from 1952, for at that time vessels for oceanographic work were made available through the Minister of Defense. So far, the University has conducted 47 cruises in the Bay of Bengal, each lasting from one day to one week. Over 700 oceanographic stations have been occupied, ranging from Madras on the south to the Swatch of No Ground on the north. Most of the work, however, is centered off the Visakhapatnam coast, as this area is especially well suited for the study of the Bay (Fig. 1).

Oceanographic cruises were carried out from Navy minesweepers by from 6 to 20 students and teachers. A cruise consists of proceeding to prearranged locations (stations), stopping the ship and lowering oceanographic instruments to measure the properties of, and to sample, the seawater. Silk plankton nets are used to filter out the small plankton organisms and dredges scoop up the benthonic forms. Sea floor sediments are sampled and the shallower depths are sounded. Some of the collected samples must be processed on shipboard in order to avoid changes in properties on storing, but the majority of data and samples are returned to the University laboratories for complete analyses. These operations are depicted in Figs. 2 and 3.

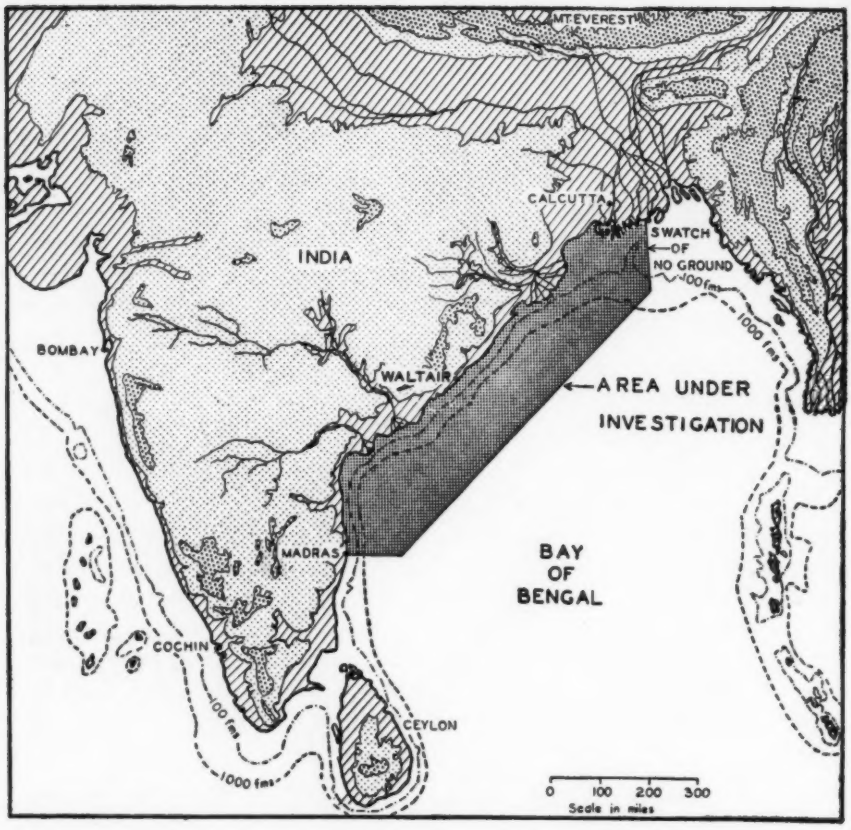


FIG. 1. Area of the Bay of Bengal currently under investigation by oceanographers of the Andhra University.

Most of the results of oceanographic studies are used for thesis material, though some are published in scientific journals and in the *Andhra University Memoirs in Oceanography*. A summary of the more important studies which have materially added to the knowledge of the area are here briefly discussed by subject—marine geology, marine zoology and physical oceanography.

STUDIES IN MARINE GEOLOGY

The marine geological studies, under the direction of Professor C. Mahadevan, deal with variations in depth of the Bay of Bengal, especially the continental shelf, and the nature of sediments of the shelf, river deltas,

and beaches. More recently the concentration and distribution of heavy mineral sands have been intensely studied, due to their scientific and economic importance.

Topography of Bay of Bengal

Deep sea bathymetry.—The sea floor topography of the central part of the Bay of Bengal is extremely simple, Fig. 4. The head of the Bay off the Ganges Delta is flat and shallow, cut only by the Ganges Submarine Canyon, or Swatch of No Ground. Below 100 fathoms the slope steepens, then decreases again very gradually, resulting in a smooth southward sloping plain with a gradient of about one fathom per mile. This uniform slope indicates that the sea floor here has been tectonically stable for a long period of time and any initial tectonic features present in this broad plain have been masked by sedimentation.

The amount of sediment discharged into the head of the Bay by the Ganges, Brahmaputra, Mahanadi and other rivers is difficult to say. One estimate is that the seaward edge of the continental shelf is steadily moving southward at the rate of one mile in 40 years.[1] Another speculation is that if the discharge during the flood season[2] contains one per cent of sediment this would amount to 4×10^9 cubic meters of sediment, or enough to cover the entire Bay of Bengal to a depth of 2 mm. every year. These, of course, are very rough estimates, because the exact figures for both runoff and sediment bedload content are uncertain. But it does give an insight into the vast sedimentation problem which exists in the Bay and its influence on the topography.

Minor irregularities.—Profiles of the southern Bay of Bengal were taken by continuous echo sounders on the Swedish deep sea ship, Albatross. In an east-west section they show that the sea floor is extremely smooth except for occasional trough-shaped depressions.[3] Five of these profiles are reproduced in Fig. 5. Their positions (A–E) lie to the east of Ceylon and are shown in Fig. 4.

The striking feature is the bilateral symmetry of the depressions. The sides are leveed like the banks of a river. It has been suggested that they are the results of turbidity currents, probably extending the length of the Bay, or 1000 nautical miles, from the Swatch of No Ground or other uncharted canyons in the head of the Bay.[3]

This process may conceivably come about by, (1) the enormous sedimentation of the rivers at the head of the Bay, and (2) a density circulation

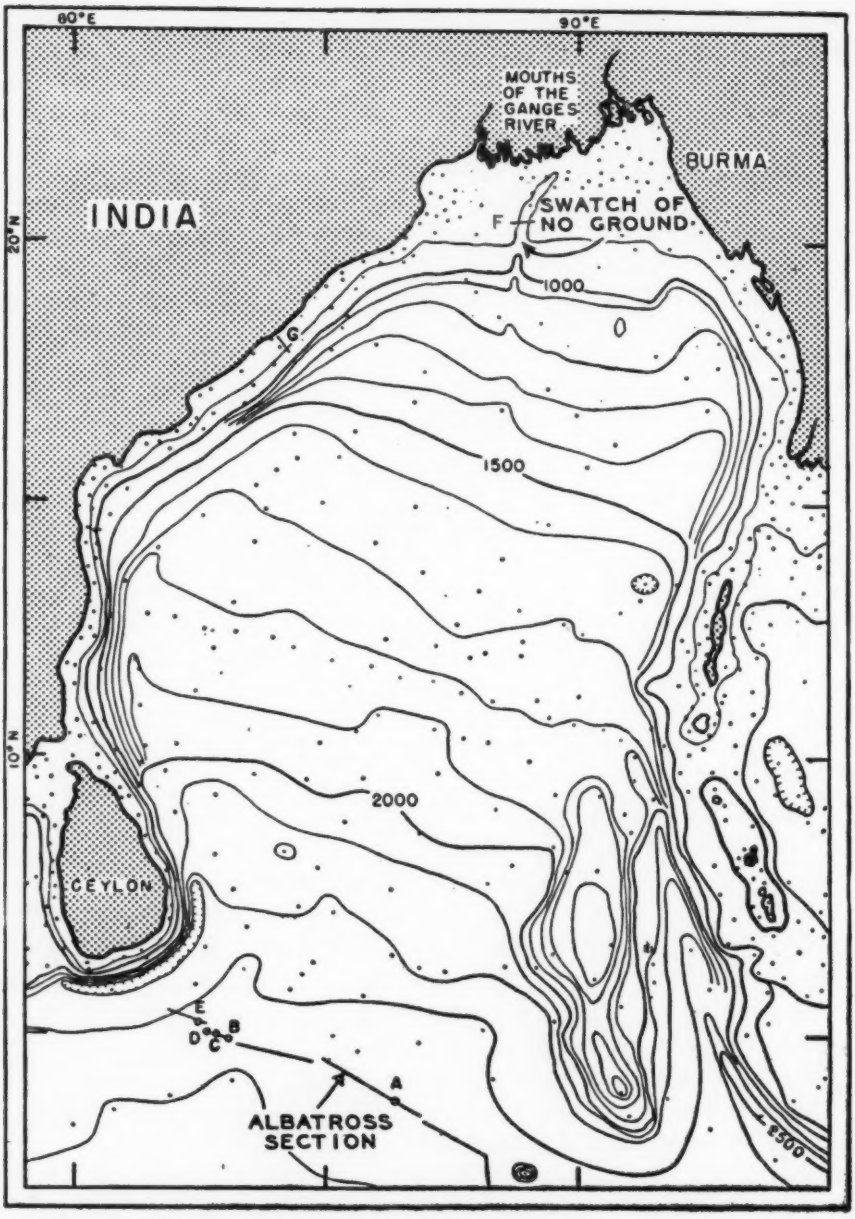


FIG. 4. Bathymetric chart of the Bay of Bengal.[8] The gradual decrease in depth to the south is shown by the nearly equal spacing of the 100-fathom interval contours. The location of detailed bottom topography (A–F) is shown in Fig. 5 and G in Fig. 6.

set up by " relaxing currents " at the termination of the upwelling period (The latter will be discussed later.)

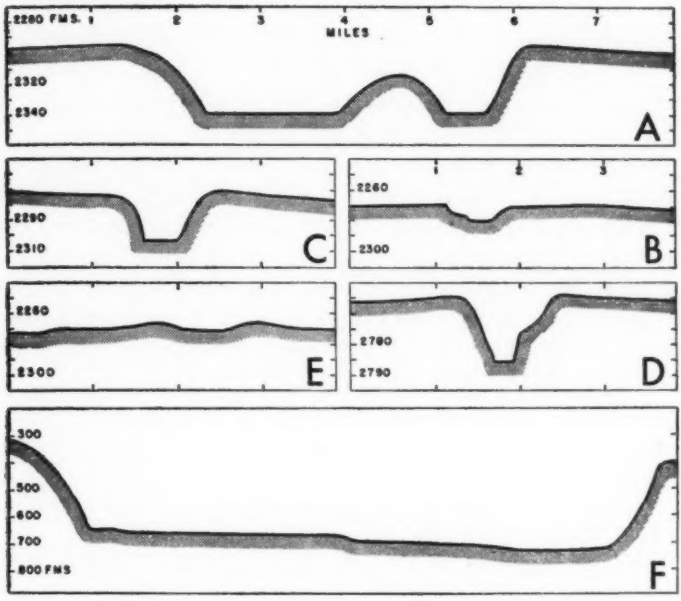


FIG. 5. Bathymetric profiles of trough-like features in the Bay of Bengal. A—E in southern part of Bay (vertical = 18 × Horizontal scale) and F across the Swatch of No Ground[4] (vertical = 3 × Horizontal scale).

Thus, the sea floor of the entire Bay appears to be a very gentle slope cut by a network of channel-like depressions running north to south.

Swatch of no ground.—A world famous feature of the Bay of Bengal is the submarine canyon with the impressive name, Swatch of No Ground. This canyon cuts the continental shelf in a general north-east—south-west direction at the northern end of the Bay.[4]

Five profiles across the canyon were made by the Danish Deep-Sea Expedition's vessel H.M.S. Galathea. One of them is represented in Fig. 5, and its approximate position is given as F in Fig. 4.

This crossing, F, shows the canyon to be about 8 nautical miles wide and about 700 fathoms deep. In this region the canyon is cut down into the surrounding plain at 3 to 400 fathoms. The slopes are steep, 1 : 1·5, but he bottom is nearly level. The width remains rather constant over a long

stretch. Eighteen miles farther south the bottom of the canyon is only 100 fathoms below the surrounding plain. Here it turns slightly to the south. Thirty-six miles south, the canyon was lost to the west, but some small undulations were found running down slope at about 960 fathoms. These are similar to the levees observed in the southern end of the Bay and might also have been caused by turbidity currents.

Continental shelf.—The continental shelf in the head of the Bay of Bengal is as much as 100 miles wide but narrows to the south. Detailed profiles have been made off the Andhra Coast[5] and a representation of these sections is shown in Fig. 6. The features of the continental shelf in this region are, (1) a width of about 25 miles, (2) an average slope of 0° 15′, and (3) a depth at the outer edge of around 100 fathoms. The unique feature is that the shelf can be divided into five zones, each having characteristic slopes and sediments. A sixth zone comprises the upper part of the continental slope. Each zone runs nearly parallel with the shore.

The average widths and slopes of the zones comprising the continental shelf off the Waltair Coast are given in Table I. The widths of the zones are somewhat variable and become a little greater towards the north, in the Bhimilipatam area.

TABLE I

Characteristics of the Waltair Continental Shelf

Zone	Width (mi.)	Slope (degrees)	Depth (fms.)
Shore A–B	2	0° 26′	0–15
B–C	11	0° 04′	15–35
C–D	6	0° 11′	35–58
D–E	2	0° 35′	58–70
E–F	2	1° 09′	70–112
Beyond F (Continental slope)	?	4° 38′	112

All zones are relatively smooth, with the exception of zone C–D, lying between 35 and 58 fathoms. Here, irregularities in the bottom are as much as two to three fathoms. These are called broken bottoms. From the

character of the slope and the breaks in the different zones it appears that the deposition or erosion of the sea floor is in turn caused by currents and water masses, which are oriented parallel to the coast. Previous sea level changes must also have played a part in this irregular formation. This is discussed in the Shelf Sediments section.

Sea Floor Sediments

Deep water sediments.—The sediments of the deep Bay of Bengal basin are characterized by their content of Globigerina ooze. This area comprises only the central and deeper parts of the deeper Bay and extends north to about 17° N.[6] Terrigenous deposits comprise the northern and shallower portions.

Samples taken on the continental slope are plastic and soft in consistency and are colored dark grey with a blue tinge. They appear to consist of clay minerals, glauconite, fine mineral grains, and an abundance of micro-organisms such as foraminifera, radiolaria, etc.[7, 8]

Shelf sediments.—The shelf sediments closely correspond to the slopes of the shelf, Figure 6. They, too, can be divided into zones.[7] And though there is some overlapping, the zones are distinct enough for definite delineations. The near-shore sediment, up to about two miles or 15 fathoms, was found to be composed of sand and was named the sand zone. The sand in this zone appears to be graded and sorted, with the fineness of the grains increasing with distance from shore. The outer limit (15 fathoms) may be taken as the limit of inshore currents and effective wave action.

The second zone, extending from about 2 to 13 miles off shore, and ranging from 15 to 30 fathoms, is known as the clay zone. Here the sediments consist of clay minerals and fine grains of sand. The fine sand continues to decrease in size and amount with distance from the coast, together with a corresponding increase in the clay fraction. Shell fragments are rare.

The next sedimentary zone is called the shell zone. It lies between about 13 and 21 miles off shore and is characterized by fragments of shell and concentrations of sand. The depth extends from about 30 to 70 fathoms, with the maximum shell content occurring between 58 to 70 fathoms. In the shell zone the sands are coarser than those found in the proceeding clay zone, or beyond 70 fathoms. This interesting zone disproves the old concept of progressive diminution of grain size with distance from the coast.[9]

The shell zone may be further broken up into a central zone between 35 and 58 fathoms, termed a rock zone. Although no rock has been dredged

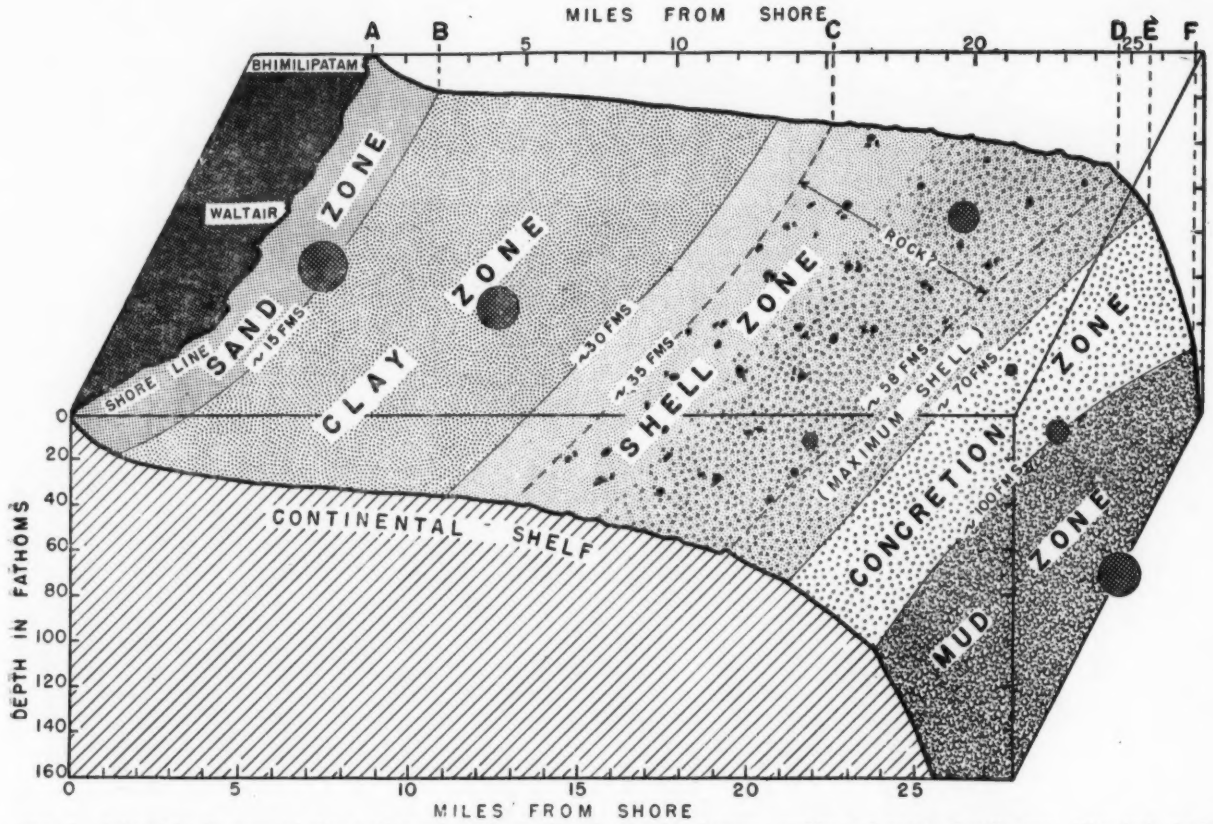


FIG. 6. Schematic diagram of the continental shelf off Waltair showing bottom profiles and zones of sediments. The dark shaded circles represent the locations where sediments were collected for radioactivity and the size is proportional to the amount.

or sampled, strong evidence points to its presence. In addition to the sharp vertical irregularities in the echo sounding record, the dredge when operating at 35 fathoms repeatedly "hung up" on hard solid objects. A sample broken off consisted of biological forms (bryozoan, sponge, hydroid and pecten, Fig. 12 J) which normally are attached to large stable rocks. Such evidence strongly implies that rock exists in this zone. It appears likely that this may have been a former beach during Pleistocene's lowered sea level, thus accounting for the coarser sediments, shells and possible rock formations.[10]

The next deeper sediment zone overlaps the shell zone and extends from 40 fathoms to the continental slope. The outer part extending out beyond the shell zone lies between 70 and 100 fathoms, roughly 21 to 23 miles off the Waltair coast. This strip is termed the concretion or oolite zone. Although the sediment in this zone is composed of clay, fine sand and shell fragments, the unique feature is that much of the material is cemented together in oval-shaped concretions or oolites. They vary in size from microscopic dimensions up to about 1 mm. in diameter. They are easily dissolved by acid, since part of the composition is calcium carbonate, iron, and other acid-soluble material. The formation of these oolites is another one of the mysteries of the Bay of Bengal.[11]

Delta sediments.—Sampling of sediments was also carried out off the Ganges, Mahanadi, Godavari, Krishna and Pennar Deltas. Though not identical, they can be discussed together. These sediments are finer than the coastal sediments, and consist mostly of silts and clays. They are generally darker (Krishna and Godavari) than the coastal samples. Another contrasting feature is the surprisingly low shell content off the deltas. A deltaic environment is apparently not conducive to the production of molluscan type organisms. The deltaic sediments, then, are the product of deposition and settling out of the finer sediments, probably facilitated by the electrolytic action of salt-water on the finer particles at the outer parts of the delta.

Radioactivity of sediments.—Samples of sediments collected along the east coast in the Bay of Bengal were also examined for their β-activity, from which the equivalent radium content was deduced.[12] On the continental shelf in the Waltair area the relative radium content of the sediments can be grouped in zonal patterns similar to those of the bottom slopes and sediments. For example, in Fig. 6 the dark circles indicate that there is a zone of relatively high intensity ($11\text{–}14\times10^{-6}$ gm. of *U.* per gm. of sediment) extending from shore out about 12 miles and to a depth of 30

fathoms. This high value was presumably caused by the presence of monozite and zircon in sediments, which are common minerals on the beach.

The second zone is composed of sediments with low values of radioactivity ($3–7 \times 10^{-6}$ gm. of *U*. per gm. of sediment) and was found between 12 and 23 miles off shore in a depth of between 30 and 100 fathoms. The low intensity of radioactivity on this former beach area has not been satisfactorily explained.

The third radioactive zone on the continental slope again contained a relatively high concentration ($13–15 \times 10^{-6}$ gm. per gm. of sediment). This high level is in accordance with other places in the world and attributed to the greater thickness of sedimentation containing thermal energy.

Beach Erosion

Beautiful sandy beaches extend all along the east coast. They derive their sand not so much from the Ganges River at the head of the Bay as from the numerous smaller rivers to the south and, to a lesser extent, from the erosion of the shore line (Fig. 1).

Sand sorting.—When the sediments are first deposited at the confluences of rivers, they consist of varying grain sizes and densities. Here at the confluences the sediments are initially sorted according to size—the coarser sands remain on the beach and the finer particles are carried off and deposited farther off shore. The beach sediments are further partially sorted according to density, whereby the heavier minerals (monozite, garnet, ilmenite, magnetite, etc.) settle out near the confluences and the lighter minerals (quartz, felspars, micas, etc.) are carried progressively further along the beach to the north and west by the predominant littoral drift (Fig. 12 A, B). From cores and magnetometer studies it was found that heavy mineral concentrates or black sands are developed near the river mouths from which they are derived.[13, 14]

Other black sand concentrates develop on open beaches where erosion takes place. This may be by a storm, during which the foreshore is eroded, or it may be caused by a long period of high waves, such as in the summer. In the process a greater concentration of heavy mineral sand remains due to the higher resistance of these to the water forces.

The greatest factors in concentrating black sands are continued erosion, or beach regression. Such conditions occur when an unnatural structure is placed in the water, such as the Madras Harbour or the Visakhapatnam breakwater. Sand from the south is trapped by the obstruction, but the sand to the north erodes, until equilibrium is reached.[15]

Another example of heavy mineral concentration is the case of Godavari Point. In its development over the past century, it has trapped the sand which formerly supplied Uppada Beach, causing the beach there to erode over a furlong, and resulting in considerable property damage.[16] This extensive beach regression, however, results in a large mineral deposition of commercial value.

Annual cycles of beach erosion.—The sand level changes on Waltair Beach were measured by a method which, though accurate, is simple and inexpensive. An eight-foot bamboo staff was marked off in feet and inches. This was used both for horizontal and vertical measurements across the beach. The latter were made by a line of sight intersections on the vertical staff between a permanent reference rock and the horizon of the sea, Fig. 7. It was found that the width of the beach does not remain fixed. Under the influence of waves, currents and, to some extent, wind and rain, the sand is moved towards land and out to sea.[17] Such movements are reflected in the sand level on the beach. Some of the movements appear to fluctuate in cycles related to the season and tides.

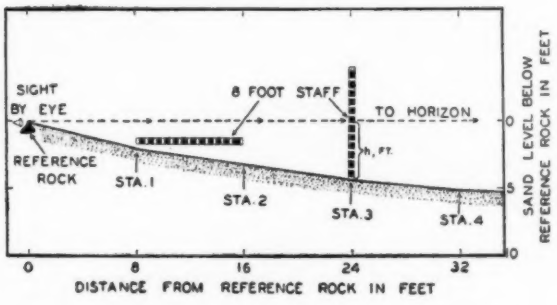


FIG. 7. Method of measuring sand level and beach profile with staff.

The seasonal sand level changes on Waltair Beach are represented in Fig. 8 together with the seasonal cycles of wind, waves, current and sand deposition for the central east coast. It can be seen that during the period of maximum waves the foreshore is eroded and the sand is deposited offshore. On the other hand, during the quiet weather period the sand "creeps" shoreward again, building up the beach. Thus, there is an annual cycle of sand levels, with the high values in March and April and low levels in October and November. The change on the Waltair Beach amounts to as much as 10 feet.[18]

Bimonthly sand level cycles.—The profile of the beach frequently varied from spring to neap tides, describing a bimonthly cycle in sand level.[19] The

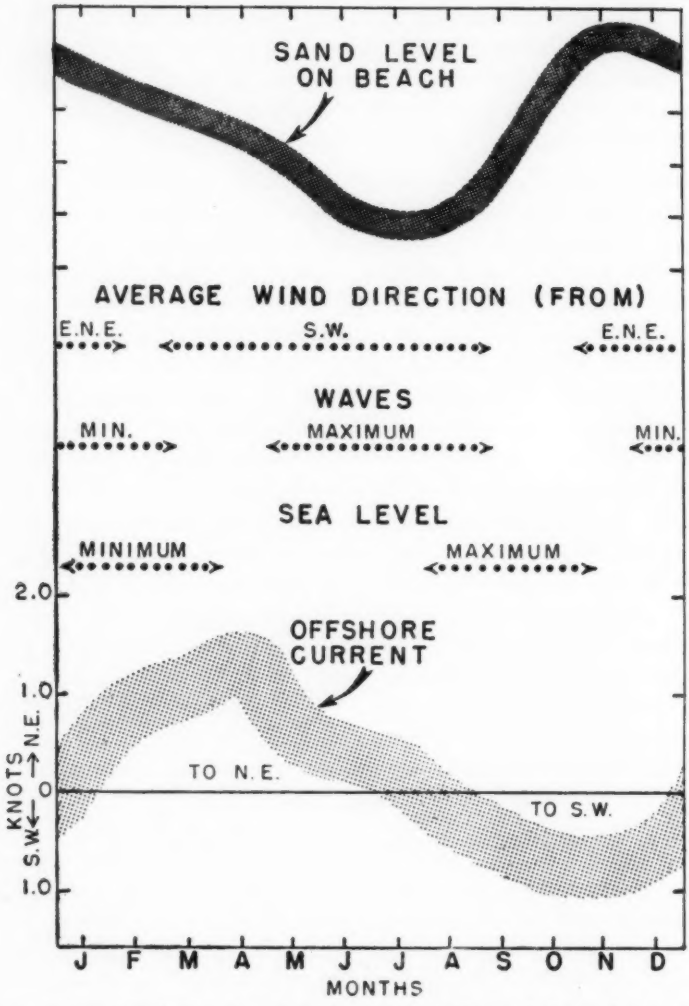


FIG. 8. Seasonal variation of sand level, sand deposition and the environmental factors affecting beach erosion.

change in observed sand levels, which took place during a series of spring and neap tides, is depicted in Figs. 9 A, B, C, and 9 A′, B′, C′. Here, too, the observed tide height and the per cent of time the beach is washed by the tide (and waves) are shown in the same vertical scale as the mean sea level.

The sand level appears to oscillate about a nodal point approximately one foot below mean tide level (C, C'). The main differences in the environmental conditions, aside from the height of water during the spring and neap tides, are (1) relative time that any vertical level of the beach is washed by the waves, and (2) the character of the waves caused by the difierence

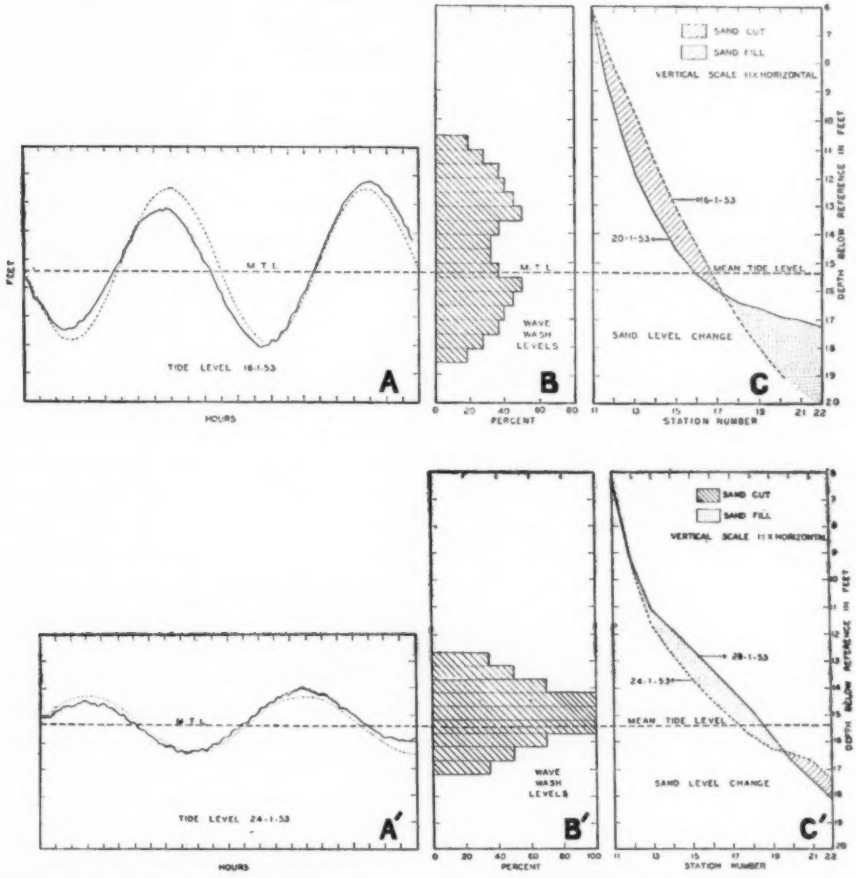


FIG. 9. Comparison of observed neap tide (A) and spring tide (A') heights in Visakhapatnam Harbour, calculated per cent of time each beach level is washed by waves during neap tides (B) and spring tides (B'), and the change in sand level during neap tides (C) and spring tides (C).

in water depth. By calculating the washed zones it was found that spring tides produce two zones of long duration wash (B), while neap tides produce

only one (B'). This is thought to be a major factor in sand movement on the beach. In addition, the wave energy at low tides is believed to be dissipated over the long shallow offshore area, whereas at high tide the energy is expended on the local zone high on the foreshore.

Slope of the beach.—Studies of the slope of the beach were made with reference to the nature of the swash and backwash. The speed of the wave as it rushes up the beach, stops, and recedes was determined with a simple manometer, as shown in Fig. 10. The maximum speed of the water, both up and down the beach, was obtained by holding this instrument in the water and reading the manometer scale. Several beaches of different slopes were examined, from which it was ascertained that a direct relationship exists between the difference in speed of swash and backwash, and the slope of the beach.[20] In other words, in order to maintain a steep beach a faster shoreward motion is required in the swash than the offshore motion in the backwash.

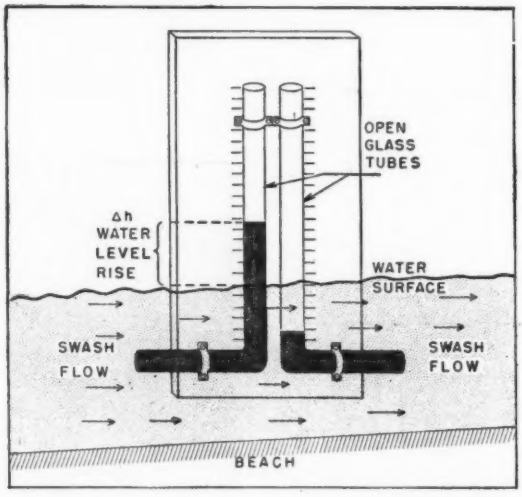


FIG. 10. Manometer to determine speed of swash and backwash.

Littoral drift of sand.—The sand on the beach acts as a fluid, slowly flowing along the beach, as well as on and off shore. The main direction of this littoral drift is to the north-east and the head of the Bay. Some reversals of drift are evident, but the environmental conditions which cause the drift (waves, currents, etc.) are more influential in a north-easterly direction. For example, it can be seen in Fig. 8 that the current flows north-easterly

for 7 of the 12 months. Its speed in that direction is also greater than when it is south-westerly. Another important factor is that the waves are higher during the north flowing current. This facilitates suspension of the sand, so that it is easily transported by the along shore currents. Still another factor is that the major waves are from the south. This is due in part to the longer fetch in the south Indian Ocean, over which the wind may blow and develop large waves.

The littoral drift of sand in a northerly direction is manifested in several ways. One is the sand bar partially across small river mouths. The bar invariably extends from the south bank, indicative of sand flow from that direction.

The development of the broad sandy beach south of the Madras Harbour breakwater is evidence of the large quantity of sand accumulated from the south. The Visakhapatnam breakwater ingeniously traps sand from the south before it fills the channel. However, the building up of Godavari Point is the best example of a northerly drift and deposition of sand.[16]

A century ago, Godavari Point was indistinguishable from other sand bars at the mouth of the Godavari River, Fig. 11. Due to floods or deforestation in the Godavari delta, more of the river flow began to discharge in the open sea rather than into Kakinada Bay. It brought with it increased sedimentation which began flowing up the coast, and depositing at Godavari Point. Over the past century the Point has steadily grown from south to north until it reached its present size. Other deposition has taken place in the southern part of the Harbour, as well as north of Kakinada jetty. Uppada Beach, on the other hand, has been eroded and its supply of new sand has been cut off by the development of Godavari Point. The deposition rate at Madras, Visakhapatnam, and Godavari Point is approximately one million tons per year—about 400,000 cubic yards. From the foregoing, it can be seen that the marine geological problems are varied and unlimited—a real challenge to students in this field.

Studies in Marine Zoology

The marine zoological program, directed by Professor P. N. Ganapati, Head of the Zoology Department, comprises studies of the marine fauna and flora of the east coast. These include plankton, as well as benthonic forms, and their relation to fish. Research problems dealing with nutrients and other chemical properties of the water, turbidity and surface appearance are also pursued.

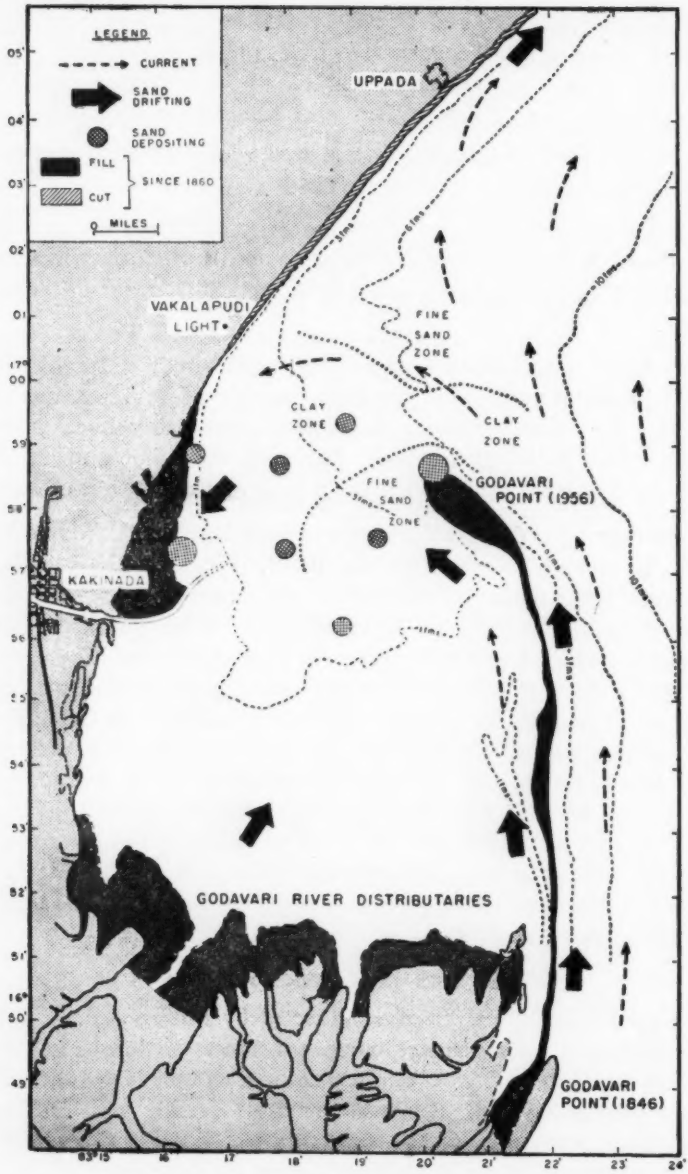


FIG. 11. Composite chart of the northern distributaries of the Godavari River, Kakinada, and Uppada Beaches (from Admiralty Chart No. 81, published in 1846 and 1860, and U.S. Hydrographic Chart No. 1711, published in 1951). Shown are the cut and fill over a century, currents, sand drift and sand depositing.

Sea Surface Features and Water Transparency

Sea surface.—Two phenomena of the sea surface, namely slicks and foam, were found to be related to the organic or biological characteristics of the water.[21] The surface water under slicks (glossy patches or streaks on a ruffled sea) was collected and examined in the laboratory. It was found to contain a high concentration of organic matter—mainly decomposing phytoplankton and zooplankton. This film produces a lower surface tension, reducing the formation of small wavelets and giving the water its glassy appearance, Fig. 12 C. The streaks are a concentration of this film caused by convergent circulation. In other words, there is a tendency towards a downward motion under the slicks, verified by a lower thermocline.

Foam, or white caps, on the open sea are caused by strong winds breaking the wave crests, entrapping air and developing foam at the surface. Sometimes this foam will persist for only a few seconds, sometimes for 20 seconds or longer after the wave breaks. The duration of its persistence correlates with the planktonic content of the water. That is, in the spring during high plankton blooms the foam will persist longer than it does in the winter. In some near-shore eddies and streaks on the sea, foam persists for hours or days, Fig. 12 G. Samples of this foam revealed a very high concentration of organic material.

Sea-water foam was also investigated by means of an electric shaker, in which different samples of sea-water were mechanically shaken and the characteristics of the resulting foam layers were examined, Fig. 12 D, E. It was found that the half life of the foam layers varied inversely with the temperature and directly with the salinity. Harbor water, water in slicks, and water of high organic content produced a thicker layer of foam that persisted longer than did normal open sea-water, Fig. 12 F.

Water transparency.—The transparency of the sea was initially made by visual observation of a secchi disc as it was lowered into the sea,[22] Fig. 3 A. Later a hydrophotometer (Fig. 3 H, J) was utilized in which light, transmitted through one meter of water to a photocell, was electrically recorded on deck. The degree of light absorbed or scattered in the water is dependent upon the nature of the material suspended or dissolved. These suspensoids may be organic, such as plankton, or inorganic, such as fine particles of sand or clay.

In the upper twenty meters of water off the east coast there are two turbidity cycles during the year.[23] In winter the water is beautifully

clear and blue, but at the onset of upwelling (to be discussed later) a subsurface layer of turbid water develops. This is believed to be caused by the rise of nutrient laden water into the euphotic zone, facilitating the production of phytoplankton (Fig. 13 A). Later, with increased upwelling,

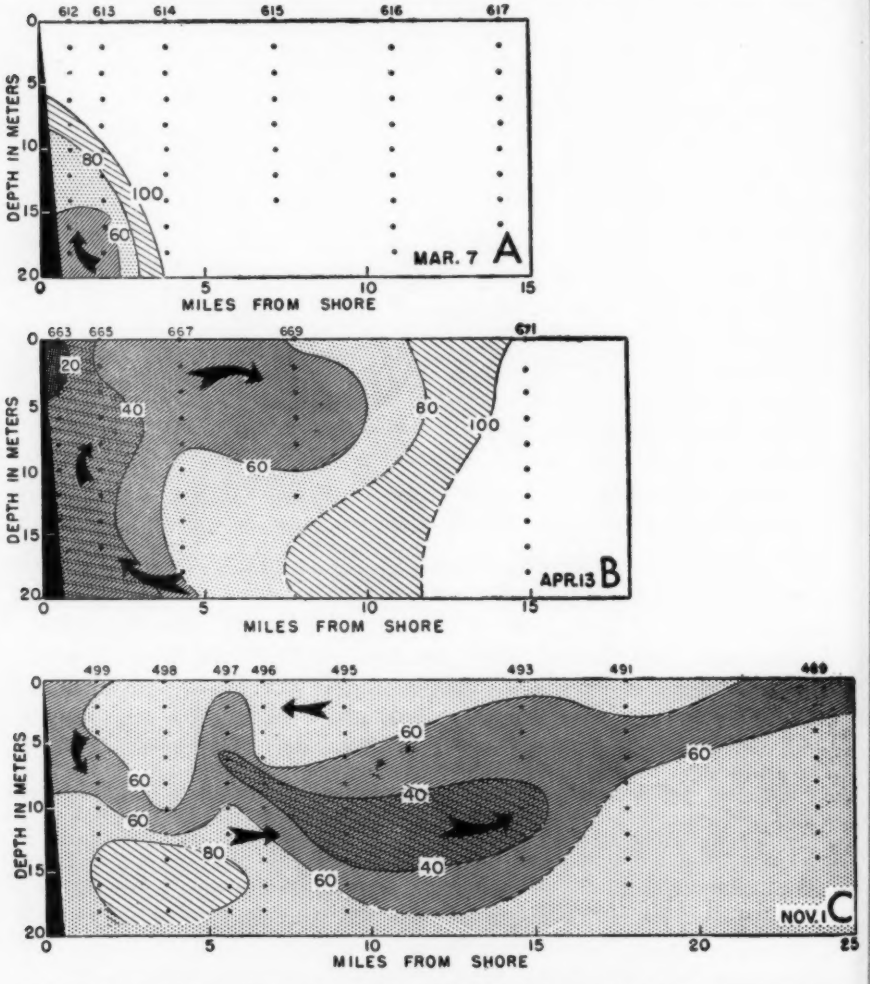


FIG. 13. Turbidity patterns and probable vertical circulation of the water in a section extending seaward from the Waltair Coast. (A) Beginning of upwelling in March. (B) Upwelling developed in April. (C) Near-shore sinking in November. (Units and shading are relative—contours represent the approximate per cent of light transmitted through one meter of water as compared to air.)

the high phytoplankton concentration or turbid zone reaches the surface. The characteristic feature of the fully developed spring upwelling is a highly turbid band of water near shore which spreads seaward at the surface and decreases with distance from shore (Fig. 13 B). In the fall, coinciding with the rainy season and runoff, another turbid pattern develops. Turbid runoff water, which is composed mainly of fine inorganic particles discharged by rivers, develops a dense layer at the surface and a very muddy layer along the botton. The latter is influenced by the downward circulation at that season. By using turbidity as a conservative property of the water it is possible to trace the circulation from these patterns. The deduced circulations are shown in Fig. 13 C.

Plant Nutrients and Other Chemicals

Surface salinity.—The general distribution of the salinity of the surface water of the entire Bay of Bengal is known.[24] The central and southern parts of the Bay are relatively uniform throughout the year, ranging from 33 to 34‰. The coastal regions and head of the Bay, on the other hand, are

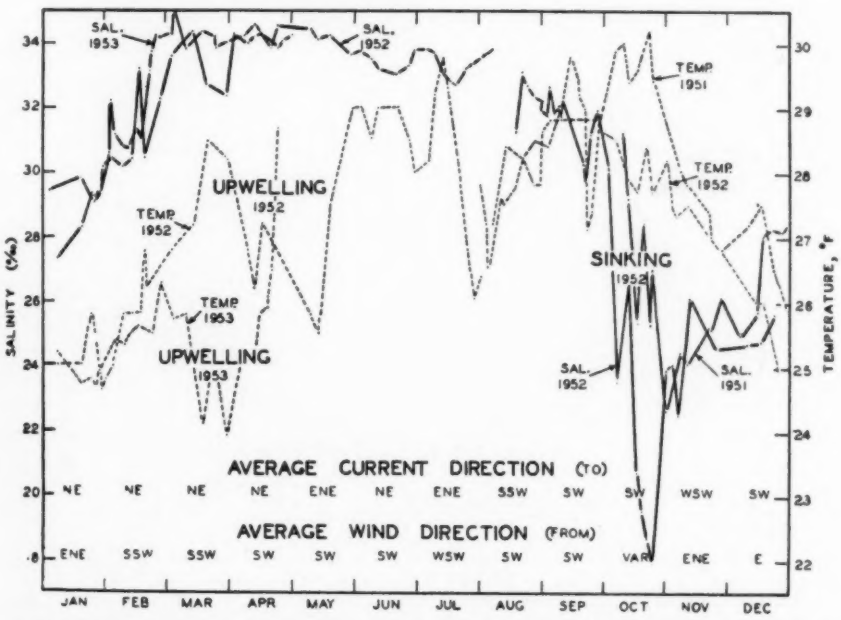


FIG. 14. Seasonal cycle of surface salinity (solid lines) and temperature (dash lines) off Waltair showing periods of upwelling and sinking, together with average monthly current and wind direction (1951–53).

extremely variable.[25] Off Waltair where most of the studies have been made the surface salinity is about 29‰ in January, increasing to 34‰ in March with the development of upwelling and the strong northerly flow,[26] Fig. 14. It decreases slightly during the summer and drops abruptly with the fall rains, even to as low as 18‰ (1952). The sinking circulation tends to confine the low values near the coast and over the continental shelf.[27]

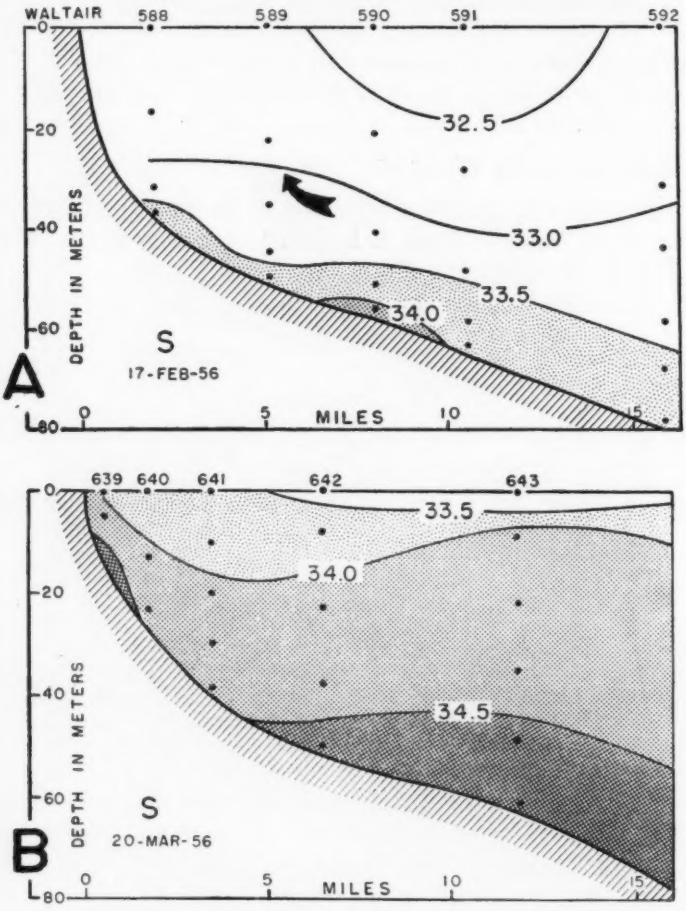


FIG. 15. Vertical salinity section across the continental shelf off Waltair at the onset of the upwelling period (1956). (A) February. (B) March. (Salinity in parts per thousand.)

Vertical salinity structure.—Vertical salinity gradients across the central shelf reflect the runoff and circulation.[28] In the fall the isohalines

are tilted upwards when going away from the coast. The thick layer of low salinity water near shore is the result of both the runoff and sinking. In spring the isohalines are tilted downward when going away from the coast, similar to those of phosphate, silicate, oxygen, temperature, etc. A comparison of the vertical salinity across the continental shelf near Waltair in February and March is shown in Fig. 15 A and B. A rapid increase takes place with the onset of upwelling and the northern flow. In spring the vertical gradients of salinity are lower with surface and bottom values

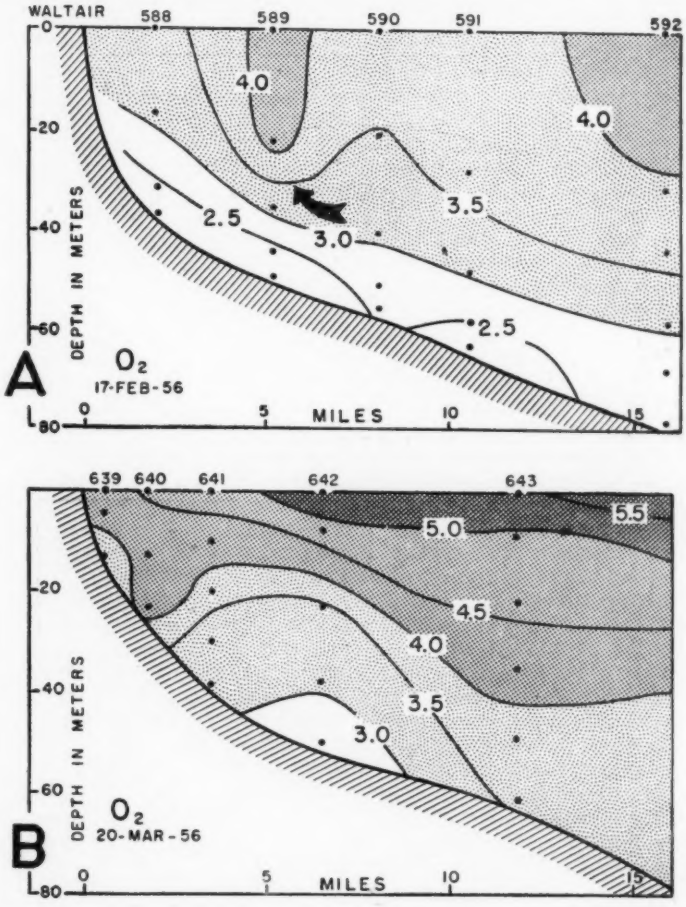


FIG. 16. Vertical oxygen section across the continental shelf off Waltair at the onset of the upwelling period (1956). (A) February. (B) March. (Oxygen in ml./L.)

of 32·5 to 34·0 and 33·5 to 34·5 over the shelf, than are those in the fall where the difference is as great at 18·0 to 34·0 (in 1952).

Oxygen structure.—The oxygen content of sea-water off Waltair varies somewhat with the season. In coastal shelf water the surface values range from 3·7 to 5·6 ml./L. in the spring and from 3·1 to 3·8 in the fall.[26] The bottom water is more uniform, 2·2 to 2·9 throughout the year. The rapid change in the vertical oxygen structure from February to March 1956 is shown in Fig. 16 A and B, and reflects the circulation of low oxygen water towards the surface and the production of oxygen by phytoplankton. The

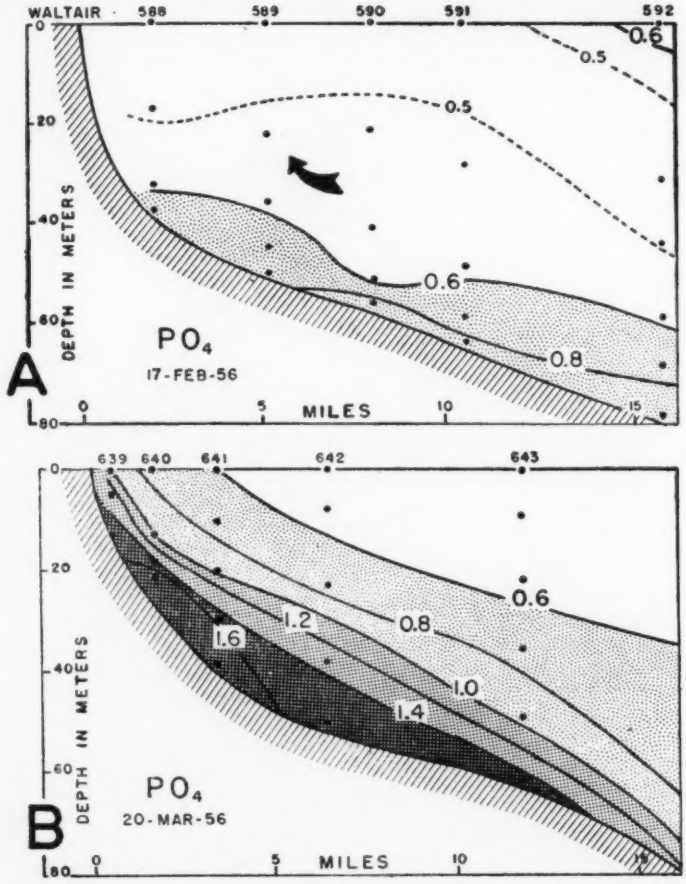


FIG. 17. Vertical phosphate section across the continental shelf off Waltair at the onset of the upwelling period (1956). (A) February. (B) March. (Phosphate in μg.-atoms/L.)

latter effect predominates and the oxygen content of the water increases about one ml./L in this one month.[28]

Phosphate structure.—Phosphate is considered one of the essential plant nutrients. With a high phosphate content and sunlight, phytoplankton are produced in abundance. A comparison of the same two months, February and March, shows the change in phosphate content of the water across the continental shelf off Waltair,[29] Fig. 17 A and B. When the high phosphate water reaches layers near the surface, as shown in Fig. 17 B, there is a large flowering of phytoplankton.

Silicate structure.—Silicate, like phosphate, is an essential nutrient salt for some types of marine plants and animals. And, as with the phosphates, its concentration is a measure of the initial growth of organisms. It can be seen in Fig. 18 A and B that the amount of silicate in the coastal waters increased greatly during a period of one month, due to upwelling and the northerly circulation. Consequently, spring silicate values are much greater than are the fall ones. As with phosphate, the greatest values are near the bottom, with isolines tilting upwards toward the shore.[29]

Marine Organisms

Plankton.—Plankton comprise an important link in the organic cycle of the sea. Near shore both phytoplankton and zooplankton collections were made from a catamaran. A minesweeper was used throughout the western Bay of Bengal for the offshore collections. The most important study in this field has been on chætognatha (arrow-worms). It was found that they bear a close relation to salinity (and other nutrients) in that they occur in greater abundance off shore when the salinity along the coast is low. Further, a study of the distribution of chætognatha in space and time has revealed the possibility of some species being considered as indicators of the different water masses.[30] Thus *Sagitta bedoti* is a common form of the inshore or nearshore waters while *Sagitta neglecta* is an indicator of shelf water. The occurrence of *Sagitta serratodentata* coincides with the incursion of oceanic waters into this area under the influence of the northerly current. Other studies on a variety of copepods bear a similar relationship with the environment.[31] For example, a near-shore peak of maximum abundance of both chætognatha and copepods occurs when the water temperature is lowered and the salinity is increased. This corresponds to the upwelling period, in which not only high salinity water is brought near the surface but also rich phosphate and silicate water as well. Some anomalies in the distribution of plankton still remain to be explained.

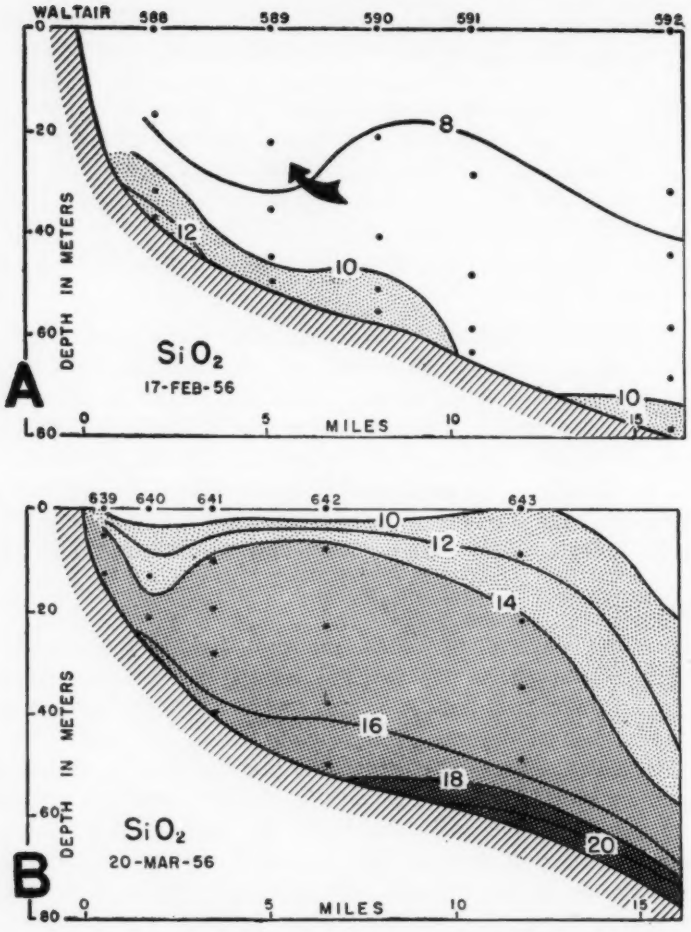


FIG. 18. Vertical silicate section across the continental shelf off Waltair at the onset of the upwelling period (1956). (A) February. (B) March. (Silicate in μg.-atoms/L.)

Benthonic organisms.—The bottom living organisms comprise the chief source of food for many fishes. To study this relationship and food it is essential to have a knowledge of their distribution abundance and the influence of the environment on these animals. Numerous dredge collections at 10 to 35 fathoms were made along the east coast of India from Puri (19° 40′ N) to Kakinada (17° 00′ N). These samples revealed the abundance of a variety of coelenterates, crustaceans, molluscs, echinoderms and a few flat fishes.[32]

From the observations made and samples collected it was found that the distribution and abundance of animals are chiefly influenced by the nature of the bottom. Three distinct types of bottom environment characterized the dredgings.

To the north of Vishakapatnam the dredgings were made on a bottom composed of a greater percentage of sand, and were dominated by the echinoderms. Some of the animals collected from this region were:

Coelenterata: *Lytocarpus* sp., *Trochocyathus* sp.

Annelida: Polychætes: tube dwelling forms, *Polydontes melanotes*, *Diopatra neopolitana*, *Hyalinœcia tubicola* and *Hesione* sp., *Armandia* sp. and *Sternaspis scutata.*

Arthropoda: Crustaceans:—*Callapa lophos*, and *Albunea symnista.*

Mollusca: *Sinum planulatum*, *Oliva oliva*, *Malius albus*, *Glycemeris tyllori*, *Solen truncatus and* developing eggs of squid *Loligo.*

Echinodermata: *Diadema* sp., *Echinodiscus biperforatus*, *Clypeaster* sp., *Ogmaster capella* and *Archaster typicus.*

Fishes: *Ctenotrypauchen microcephalus*, *Platyphyrys pantherina*, *Pseudorhombus* sp., and *Plyatycephalus insidiatrix.*

The form of particular interest from this region is *Diadema* sp. dredged off Kalingapatnam and Waltair. See Fig. 3 F.

The bottom south of Vishakapatnam towards Kakinada on which dredgings were made was largely an admixture of sand and mud and seems to be equally as productive as the preceding one. This region has a rich representation of coelenterates, crustaceans and molluscs. Echinoderms are sparsely distributed in this region. Coelenterates from this region were *Cavernularia* sp., *Sphenopus marsupialis*, *Flabellum pavonium* and *Gorgoniss.*

Dredgings beyond the 5 fathom line off Kakinada Bay had a surprisingly poor representation of animals, in comparison with either of the preceding areas or to the intertidal region of the Bay. For example, the only bivalve mollusc collected here was *Paphia textile.* The bottom in this region is composed of loosely packed fine sediment. This seems to be a striking example of the great influence that the oceanographic environment has on the distribution of the benthonic animals.

Fouling.—Fouling organisms in tropical waters are notoriously abundant and Visakhapatnam Harbour is no exception. Here the inevitable barnacles and serpulids (tube worms) are the chief foulers, followed in order of abundance by hydroids, amphipods, encrusting and erect bryozoa, simple

and compound ascidians, bivalve molluscs, errant polychætes, anemones, and sponges. The peak of organism abundance varied with the season, barnacles flourishing in April and May and serpulids in January and February.[33]

Fisheries.—Although the actual study of fish populations has not been pursued at the University, the zoology studies are pertinent to fish production. From a food standpoint the upwelling circulation is favorable in that it supplies nutrients for phytoplankton. These in turn supply the zooplankton, etc., which, with the benthonic population, should offer sufficient food for abundant commercial fisheries.[34]

It may be pointed out here that even a cursory examination of the available data on the fish statistics reveals a great discrepancy in the total output between the two coasts of India. An analysis of the data collected during the year 1952 revealed that the total quantity of marine fish landed in India was about 500,000 tons and of this the west coast contributed 82 per cent.[35] An investigation into the causes of this discrepancy is an important marine biological problem and it is quite likely that there are vast areas of rich fisheries elsewhere in the Bay of Bengal as yet untapped.

It is possible that the high dilution in the fall may be detrimental to some species.[36] The Central Marine Fisheries has set up a unit in the Zoology Department to study this interesting and vital problem.

Studies in Physical Oceanography

Physical oceanographic studies have been carried on under the leadership of Professor S. R. Savur, Head of the Geophysics Department, and are comprised of studies of the physical properties of the water such as its temperature and density. They also include investigations of water movements—currents, turbulence, tide levels, waves, slicks, sea-level, upwelling and sinking, as well as related meteorological factors. Physical studies are important to all branches of oceanography in that many of the biological, geological and chemical properties are influenced or controlled by the physical processes.

Sea Temperature and Density

Surface temperature.—The physical property most easily measured is the sea surface temperature. Generalized overall Bay of Bengal charts of surface temperatures show that the offshore areas are very uniform—with decreasing temperatures towards the north, and in the winter.[37]

Near-shore temperatures, like salinities, are more variable.[27, 28] Examples of seasonal cycles of temperature and salinity are given in Figs. 19

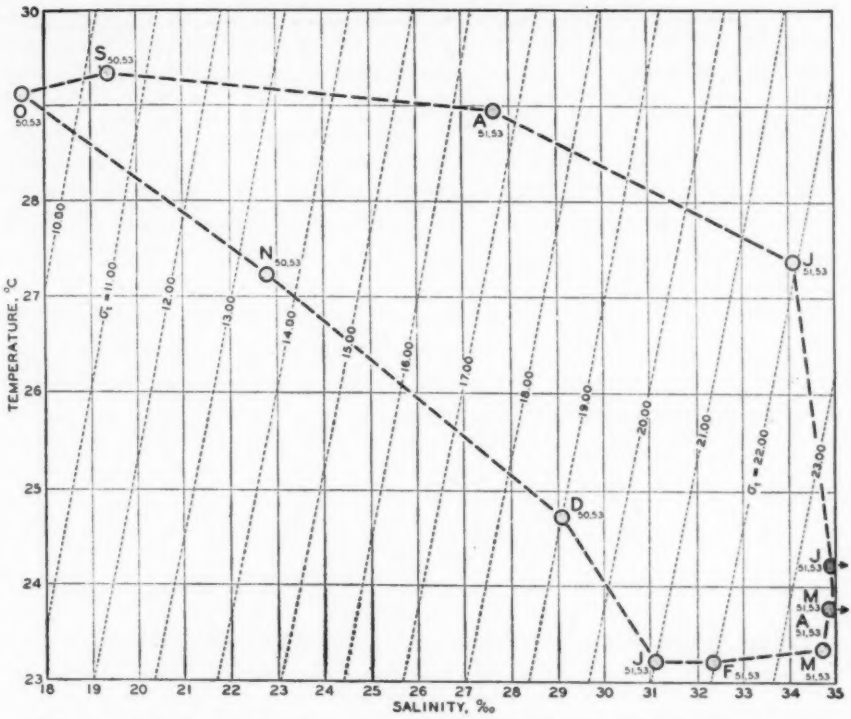


FIG. 19. Average monthly sea surface temperature, salinity and density (1950–53) at Saugor Island.

to 21. These are the average monthly values for three coastal stations —Saugar Island, Waltair and Mandapam. Monthly temperatures are plotted against salinity. Each monthly value is labeled with the first letter of the month. It can be seen that the seasonal range of surface temperature is still only 4 to 6° C. The salinity, especially in the north, reflects the influence of the fresh-water runoff in the fall season. These are discussed more fully under water masses.

Vertical temperature.—The vertical temperature structure of the sea can frequently be used to determine the physical processes going on in the sea.[39] Figure 22, for instance, gives six examples of the types of temperature-depth structure found in the Bay of Bengal.[40] The curve labelled A is isothermal water, or water that has been thoroughly mixed to the lower level of the trace. Curve B is typical of the usual three layer system (thermosphere, thermocline and psychrosphere) and the higher temperature at the surface

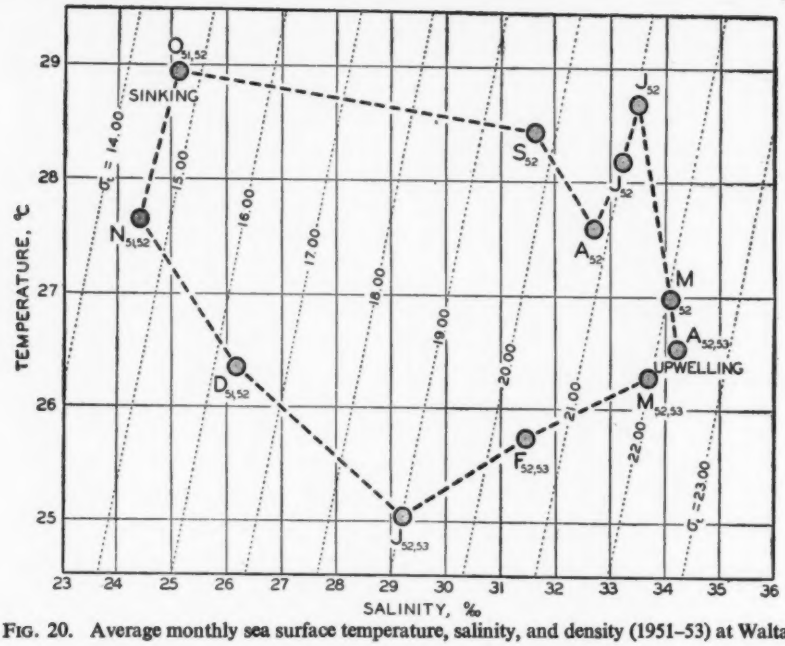


FIG. 20. Average monthly sea surface temperature, salinity, and density (1951–53) at Waltair.

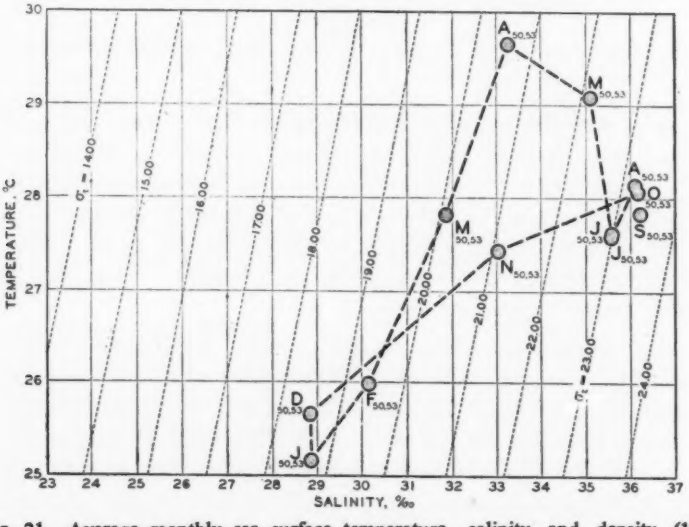


FIG. 21. Average monthly sea surface temperature, salinity and density (1950–53) at Mandapam.

represents conduction of heat to the water from the atmosphere under low winds. The step-like structure in C is usually the result of successive surface heatings and wind mixings, common in the spring. A sharp break in the bathythermogram, as in D, occurs when a water mass of a given temperature flows over another of a different and lower temperature. The boundary between the two masses remains sharp until later diffusion and mixing takes place. A strong wind may also mix the upper layer and produce a similar break in the curve. Irregular wiggles shown in E are the result of turbulence at strong current boundaries. An increase of temperature below the surface is usually the result of cooling at the surface. The surface layer under these conditions must contain water of low salinity in order that the vertical column may remain in stable equilibrium.

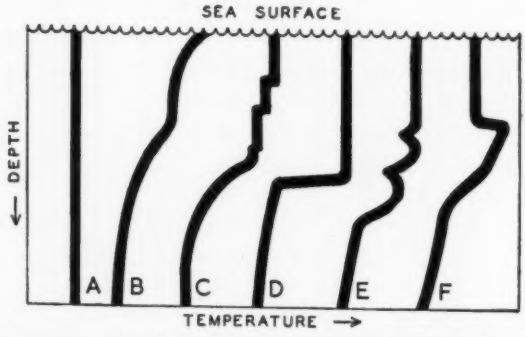


FIG. 22. Various types of bathythermograms observed in the Indian Ocean.

The most informative features of the vertical temperature structure of the water off the Waltair coast are those caused by upwelling and sinking. During upwelling the thermocline becomes weaker and rises towards the surface.[41] During sinking the thermocline becomes sharp and deeper—sometimes intersecting the bottom at the outer part of the continental shelf. This produces nearly isothermal water over the inner part of the shelf during fall and winter. All of these temperature patterns are clues used to solve the puzzles of circulation, mixing, and heat transfer in the sea.

Density and water masses.—Density is a function of temperature and salinity, and for convenience is expressed as σ_t, where $\sigma_t = (\delta - 1) \times 10^3$. By means of these T–S relations, water masses may be established which can be used to trace the direction of flow and other dynamic processes that occur in the ocean. In Figs. 19 to 21 the east coast surface density is also illustrated by diagonal dashed lines.

From the preceding T–S plot in Fig. 20, as well as from other subsurface T–S plots, it has been possible to assign names and σ_t limits to water masses for the purpose of discussing their distribution and circulation.[42] For example, water of low density ($\sigma_t < 19$), which occurs off Waltair, from October to December has been called *Northern Dilute* water, Fig. 23. A *Transition* water mass between $\sigma_t = 19$–21 encompasses January to February and August to September, and water with σ_t values of 21–22 has been called *Southern Bay of Bengal* water. The *Upwell* water mass has a σ_t of 22–23. Water having a σ_t greater than 23 is most always a subsurface water mass, therefore, σ_t of 23–24 has been called the *Subsurface Shelf* water mass. With greater density ($\sigma_t > 24$) it may be considered part of the *Indian Equatorial* water. These σ_t limits and the names assigned were based on distribution, origin and formation.

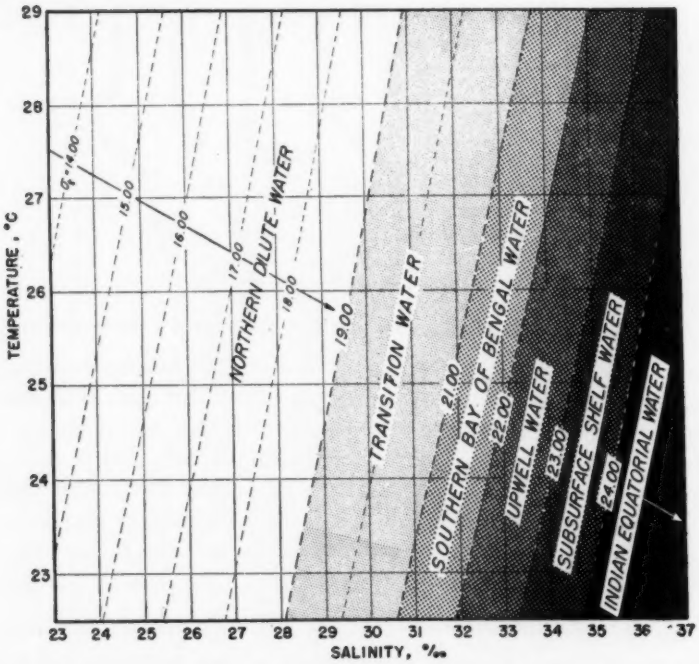


FIG. 23. Temperature-salinity diagram and related sigma-*t*. Shallow water masses for Bay of Bengal are named and assigned sigma-*t* limits as indicated by the shading.

From the observed temperature and salinity in the western Bay of Bengal, the horizontal and vertical distribution of density and water masses were determined for the months of October and March. These are illustrated in

Figs. 24 (October) and 25 (March). The area considered comprises two wedge-shaped sections running up and down the coast from Waltair, Fig. 24 A. The central part, Fig. 24 C, is an enlarged vertical section extending 30 miles out from Waltair. In the horizontal section, B of the block diagram, the scale at the northern end is compressed, as it is for the southern end of D.

On the horizontal and vertical sections (B, C, D) the water masses defined above have been represented by shadings. The lighter the water, the lighter the shading. In Fig. 24 B (October) the sea surface is covered with *Northern Dilute* water. It is formed in the northern end of the Bay by the mixture of runoff water with the more dense Southern *Bay of Bengal* water. River discharge into the Bay of Bengal is estimated at 4×10^{11} cubic meters of fresh water per year.[1] This mixture spreads out over a large portion of the north-eastern Bay and flows down the east coast.

At this time of the year all the above-defined water masses are present over the continental shelf, Fig. 24 C. The *Northern Dilute* ($\sigma_t < 19$) extends down to about 150 feet near the coast, whereas 30 miles off the coast it reaches only half this depth. The others are found as thin layers below the surface.

To the south the *Northern Dilute* water continues at the surface nearly to Ceylon, Fig. 24 D. However, its thickness becomes less with distance from its source.

The same projection is used in Fig. 25 to illustrate the distribution of water masses during the spring season. The surface densities are considerably greater in spring than in fall. The surface water mass is largely *Southern Bay of Bengal* water ($\sigma_t = 21$–22). This water mass is most always found in the central and southern part of the Bay. By spring it has now been transported up the coast by north-east flowing currents produced by the prevailing wind system which, in March, is mainly from the south-west.

The unique feature of the surface density is the band of *Upwell* water along the coast. Near Waltair it is initially only a few miles wide in March but increases by May to cover about half the width of the shelf. Off the Orissa Coast this width may be even greater.

At this season no *Northern Dilute* or *Transition* water is present off Waltair. The other water masses present over the shelf are in thicker layers than in the fall, thus making the vertical gradients weaker.

To the south some *Upwell* water extends to the neighborhood of the Godavari Delta but most of the surface is covered with *Southern Bay of Bengal* water.

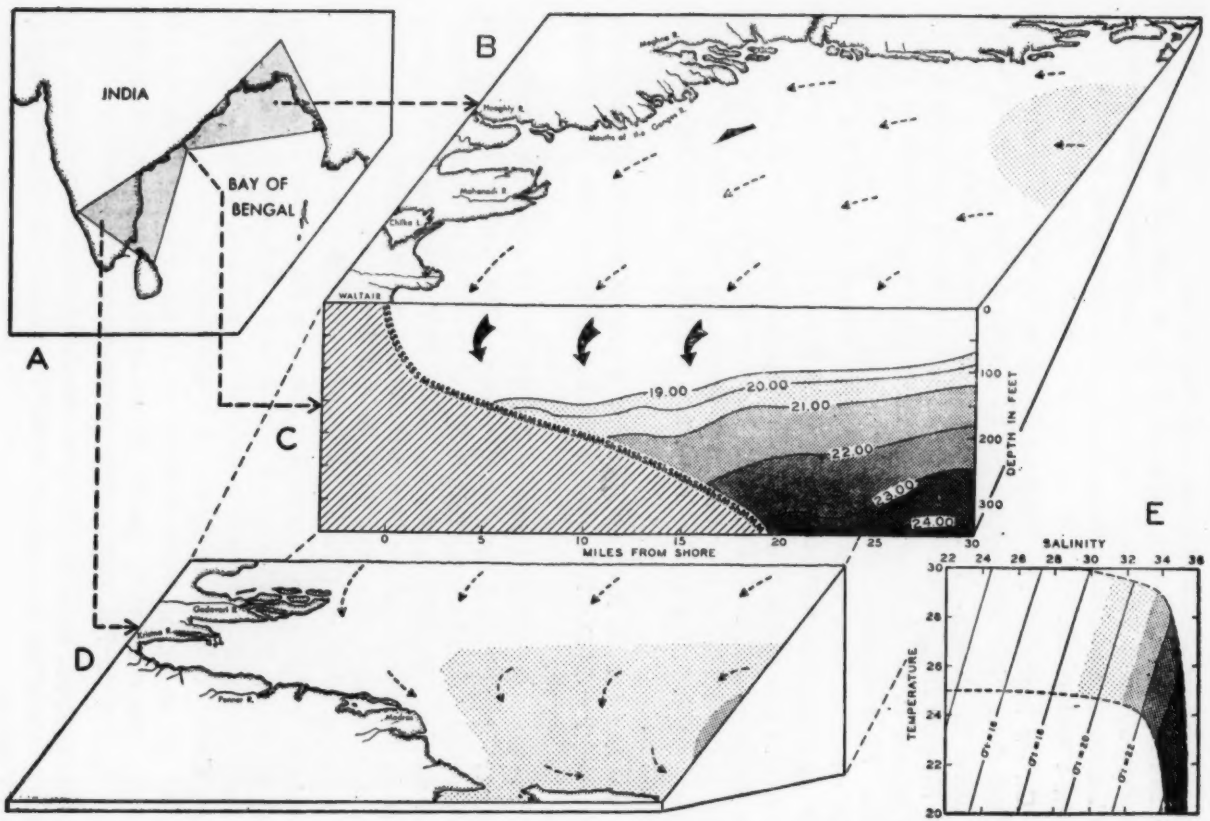


FIG. 24. *October* water masses (density distribution) and currents off the east coast of India (1952). (A) Areas used for projection. (B) Sea surface north-east of Waltair. (C) Vertical section off Waltair. (D) Sea surface south-west of Waltair. (E) T-S relations used for the water masses.

(B) Sea surface north-east of Waltair. (C) Vertical section off Waltair. (D) Sea surface south-west of Waltair. (E) T-S relations used for the water masses.

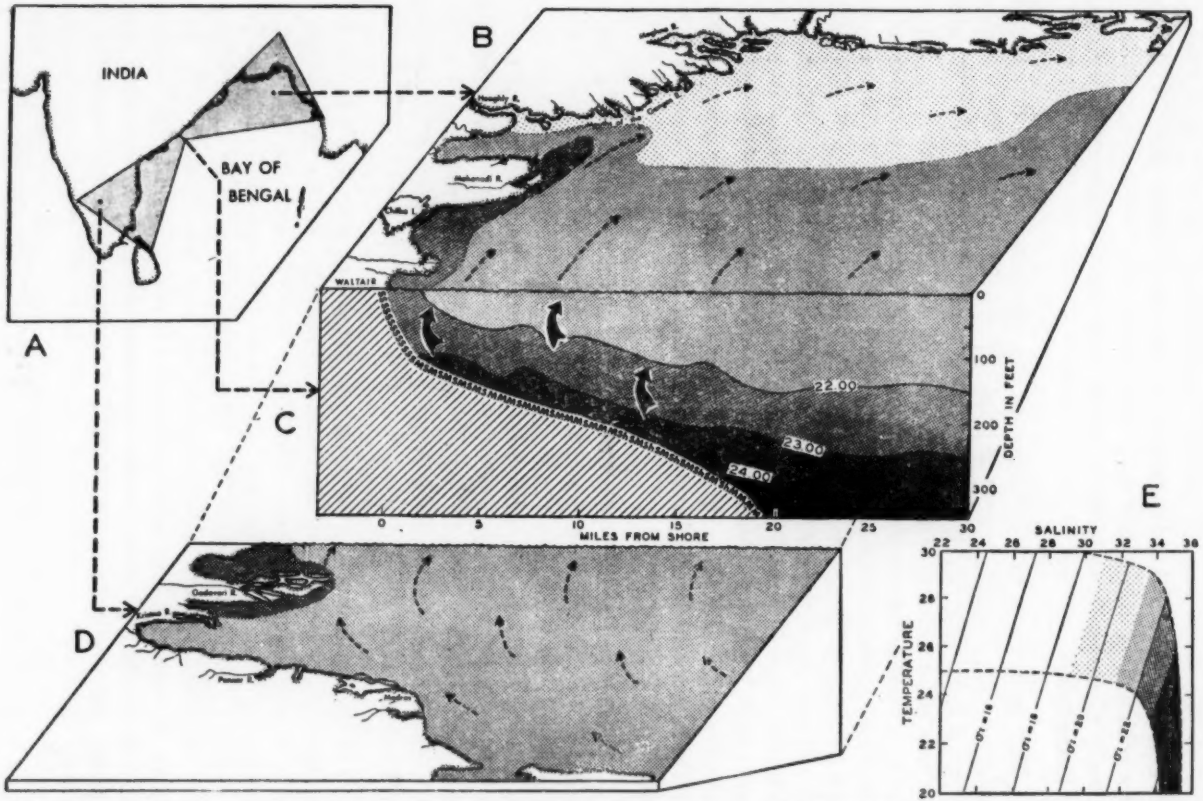


FIG. 25. *March* water masses (density distribution) and currents off the east coast of India (1953). (A) Areas used for projections. (B) Sea surface north-east of Waltair. (C) Vertical section off Waltair. (D) Sea surface south-east of Waltair. (E) T-S relations used for water masses.

Water Circulations

Currents.—From the distribution of the water masses described above, the circulation of entire Bay may be deduced. The surface circulation is generally clockwise from January to July and counterclockwise from August to December.[37]

This generalized circulation along the western part of the Bay is illustrated by dashed arrows in Figs. 24 and 25. During the transition periods it is believed that large eddies break off from the major flow patterns. The seasonal coastal flow in the vicinity of Waltair is shown in Fig. 8. This is purposely represented by a broad band because of the variability of speed, and sometimes direction. If the wind is strong for a few consecutive days its effect is manifested in the flow. However, currents to the north-east not only persist longer but flow at great speed. At times the currents off Waltair were observed to be as much as 3 knots to the north-east and off Sacramento Shoal (Godavari Delta) to be as much as 5 knots. Great quantities of *Southern Bay of Bengal* water are consequently advected from the south, only to be displaced a few months later by the *Northern Dilute* mass from the north.

Upwelling and sinking.—One of the most unique features of the Bay of Bengal is the fact that both upwelling and sinking exist off the east coast.[24] Upwelling is the process in the sea whereby the subsurface layers move up towards the surface. The reverse is possible when surface water is concentrated or piled up, usually against the coast, Fig. 24 C, thus displacing subsurface water downwards and causing sinking. The phenomena are not as extensive as observed on the Arabian Coast and are here confined to the inner shelf area.

Off the east coast the wind systems associated with the N-E and S-W monsoons not only blow the water along the coast but displace it offshore at different seasons. The forces involved and the direction of motion can be measured by the density distributions shown in Figs. 24 C and 25 C. Another relation has been developed whereby the south-west wind component bears a high correlation with the salinity.[43] In other words, the water (of high salinity) upwelled is proportional to the wind force which blows the low salinity water north and east and to the rainfall, which replaces the low salinity water. Off Waltair an empirical expression for salinity is

$$S = 32{\cdot}59 + 0{\cdot}35 \text{ wind force} - 0{\cdot}0016 \text{ rainfall.}$$

Other effects related to upwelling are shown in Fig. 14 where the sudden drop in surface temperature indicates that cold layers have upwelled to the surface. Sinking is indicated by a period of high temperatures in the fall. The best indication of upwelling is shown in Fig. 23 C, where the isopycnals are tilted upwards towards shore. Further evidence of this upwelling is the presence of weaker vertical gradients. Still other effects of upwelling are shown in Figs. 17 and 18, where the chemical properties of the water are changed by the rise of high nutrient water towards, or to, the surface. These high nutrients can come only from subsurface levels, since the surface nutrients to the south from which the current is flowing, are lower.

The old axiom of " what goes up must come down " applies to the dense upwelled water near shore. It not only goes down, but down below a neutral plane, causing sinking. The S-W wind forces on the sea, which were partly balanced by the tilting of the density field, reverse in the fall. Consequently, the density field must also reverse. During sinking along the coast the isopycnals are tilted downward towards shore as shown in Fig. 24 C. Downward motion is further brought out by the compactness of the isopycnals. Since this water displaced shoreward and downward in surface water, its nutrient content is low.

The change-over of wind systems takes place in September and October (Fig. 8). When the strong S-W winds cease blowing, the forces maintaining the horizontal unstable density distribution terminate, and a " relaxing " current is set up. The dense water near shore will gradually flow down the slope. Its speed must be very slow but it takes place along the bottom and in a seaward direction. Any depression such as the Swatch of No Ground would funnel and speed up the flow. At the head of the Bay it is conceivable that surges in these relaxing currents are strong enough to influence the down slope flow of newly deposited sediment. This might possibly be the trigger mechanism for turbidity currents which may flow the length of the Bay.

Since the general circulation at the head of the bay is counter clockwise and the relaxing current is down slope the resultant combination would be a southwesterly flow as in the direction of the Swatch.

Another interesting phenomenon related to the upwelling is an unusual isothermal temperature layer which develops at the bottom over the central part of the shelf.[44] The mixed water first appears in February as a thin layer 5 to 10 feet thick along the bottom. It thickens until by March it is 5 feet thick seven miles off shore, Fig. 26. The water has a slight

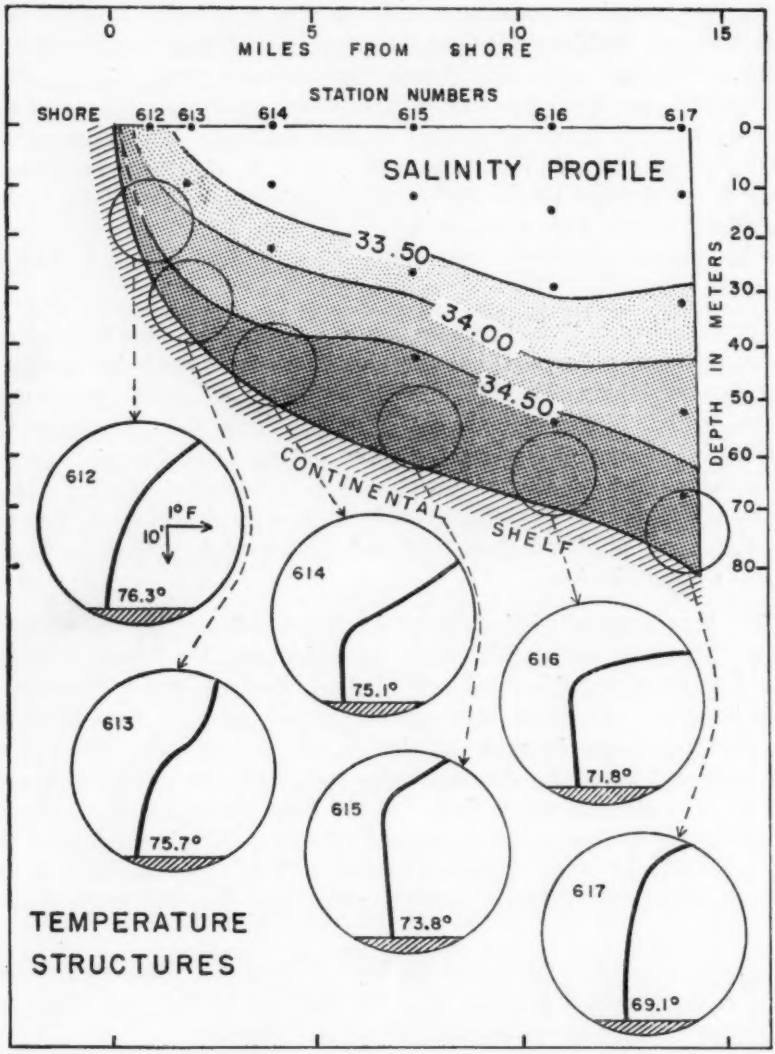


FIG. 26. Salinity profile and vertical temperature structures of the coastal waters off Waltair (March 7, 1956). Upper part: Salinity profile over the continental shelf indicating the upward tilting of isohalines towards shore produced by upwelling. Lower part: Enlarged temperature structures showing the virtually isothermal bottom water found at the indicated locations near the bottom along the central part of the continental shelf.

negative gradient near shore and at the outer edge of the shelf. The maximum mixed water is found where the water depth is between 30 and 45 fathoms. Although the water is nearly isothermal vertically, it possesses horizontal gradients. The bottom temperatures increase shoreward, as shown by the numerical values in the insets. This unique formation of an isothermal layer at the bottom seems to be caused by two agencies. The first is upwelling, which introduces water of nearly the same density and perhaps creates instability in a layer near the bottom. The second, and the more important, is turbulent mixing resulting from relatively strong currents flowing over a rough sea bottom (probably rock or coral). From the location and thickness of the layer, the position of maximum along-shore, bottom currents on the continental shelf might be deduced. Again, this is another of the inexplicable characteristics of the Bay of Bengal.

Sea-level.—In addition to water-level changes due to waves and tide' the average sea-level itself varies throughout the year. To obtain the mean sea-level the hourly tide heights are averaged over any given period. For example, the daily mean sea-level (average for 24 hours) from the conveniently located tide gauge in Visakhapatnam Harbour is shown in Fig. 27. During 1950 it was low in February and March and high in June and again in the fall.[45] However, August was low. The range in this annual cycle is surprisingly high—and the highest in the world is found in the Bay of Bengal. The oceanographic and meteorological factors suspected of contributing to these changes were examined—namely, atmospheric pressure, wind, rainfall, density and currents.

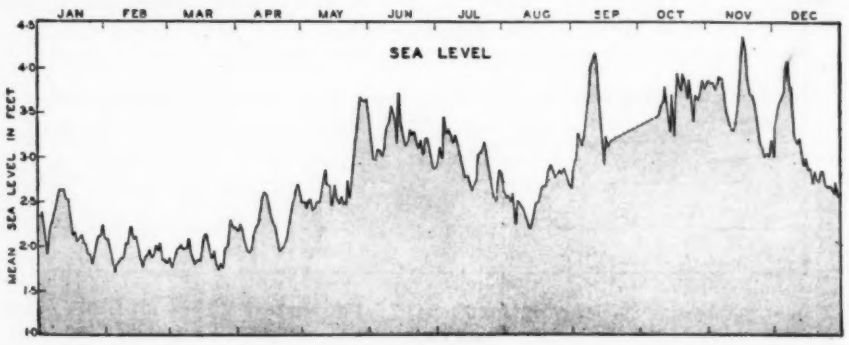


FIG. 27. Mean sea-level in Visakhapatnam Harbour for each day for the year 1950.

By calculating the dynamic heights (0/200 meters) based on the density of the vertical water column, it was found that the difference between April

and October was 0·548 dynamic meters, or 22 inches. This, then, will account for approximately 90% of the annual change. In addition, the atmospheric pressure difference between Visakhapatnam and the opposite side of the Bay of Bengal was found to account for 2 inches more of water-level, or approximately 10% of the observed range in sea-level. The other factors could not be evaluated and apparently are not in themselves significant. However, the drop in sea-level in August still remains a mystery—possibly a low rainfall in that month?

Seiches.—One feature brought out in the tide records is small waves superimposed on the smooth semi-diurnal tidal cycles. A few are illustrated in Fig. 28. They are usually more pronounced when the tide range is

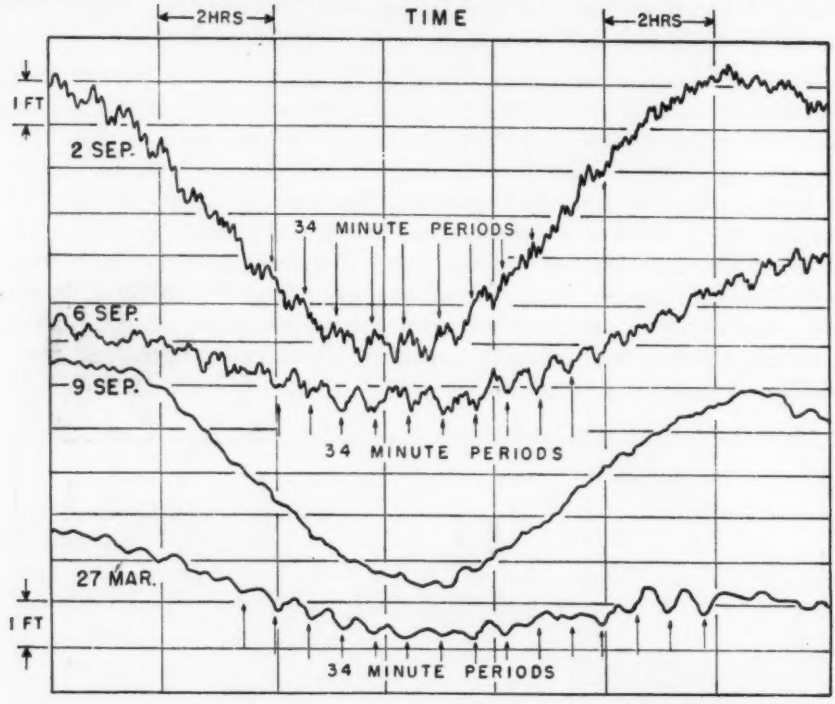


FIG. 28. Parts of the tide record for the days 2,6, 9 September and 27 March 1950, showing 34 minute seiches and other smaller vertical oscillations superimposed on the semi-diurnal tide.

low. Their heights vary from 2–6 inches and they have a surprisingly consistent period of 34 minutes.

This 34-minute period is too long for a harbour oscillation which should be about 8 minutes. Also, if a standing wave oscillated from the

harbour to the edge of the continental shelf, the period should be 64 minutes. The wave must then have some other dimensions, which makes the 34-minute seich another enticing mystery.

Internal waves.—Vertical oscillations in the sea known as internal waves, also exist in the Bay of Bengal. These were investigated by means of repeated bathythermograph lowerings at single locations and revealed temperature fluctuations with time.[46] The oscillations, when taken over a long period of time, appear to have a semi-diurnal cycle, especially at the thermocline. The most promising force creating these internal waves was that of the tide, consequently harmonic analysis was performed on two sets of repeated temperature data collected at the edge of the continental shelf. The depth of an isotherm, with respect to tide, in the thermocline of each of the sets of data can be expressed as follows:

I.O.1, and
$$Z_{820} = C_0 + 12{\cdot}8 \cos(\theta - 51) + 9{\cdot}2 \cos(2\theta - 103)$$

I.O.2, where
$$Z_{780} = C_0 + 8{\cdot}6 \cos(\theta - 91) + 16{\cdot}4 \cos(2\theta - 12)$$

Z = depth of isotherm, C_0 = constant and θ = phase angle.

It was found that the phase lag of the second harmonic, corresponding to the semi-diurnal tide, was $3^h\ 31^m$ for one case and $-\ 2^h\ 31^m$ in the other case, or completely out of phase. These data when plotted with other similar data taken in the Atlantic and Pacific Oceans indicate that although an internal wave of tidal period exists, the phase lag between surface and internal tide varies widely, Fig. 29. The majority of the 17 sets of data examined show that the "internal" high tide usually occurs 3 to 5·5 hours after the surface high, or $\alpha_{IHT} = 4{\cdot}5^h + \alpha_{MHW}$.

Shallow internal waves.—If the internal waves described above are of short periods and the thermocline is nearly at the surface, they produce a surface disturbance. The crests of the shallow internal waves ruffle the surface as they move along. The troughs, on the other hand, tend to smooth the surface, producing a slick band. Thus, the sea becomes a series of slicks and ruffled waves over shallow internal waves as shown in Fig. 12 I.

This phenomenon has been observed all along the east coast shelf.[47] On one occasion the thermal structure was examined in detail by repeated bathythermograph lowerings. From the measured thermal structure and the drift of the ship, the circulation in the zones was deduced as shown in Fig. 30. It was concluded that the wave motion was cellular in nature.

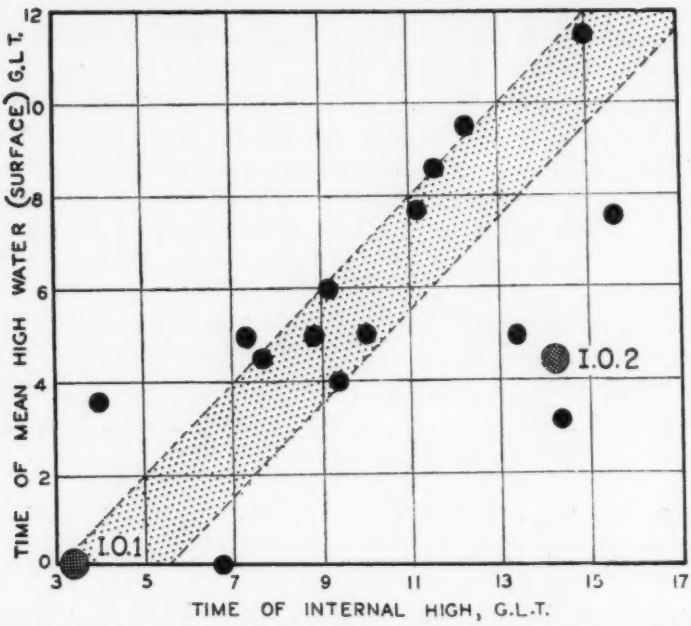


FIG. 29. Relationship of time of surface high tide and internal high tide (shaded band) for 15 stations in Pacific and Atlantic Oceans (dots) and 2 in the Indian Ocean (I.O.1 and I.O.2).

Consequently, they were termed rotary currents. The motion was further substantiated by the nature of the bathythermograph traces in which layer depths were both deeper and sharper in the trough than on the crest of the waves—a feature characteristic of sinking or downward motion. In other words, the rotary currents are a wave motion consisting of zones of convergence and divergence. They appear to develop in the turbulent region where the offshore tidal currents come in contact with the prevailing coastal currents. The development and maintenance of this boundary comprises another of the many complex phenomena of the Bay of Bengal.

CONCLUSIONS

The preceding review points out the progress that is being made in studies at Andhra University. It reveals that the secrets of the sea are many and varied.

It is probably apparent from these studies that an interrelation exists between the various fields of oceanography. For example, it is not possible to discuss plankton population or beach erosion without reference to currents and waves. All are interrelated. Consequently, the solution of

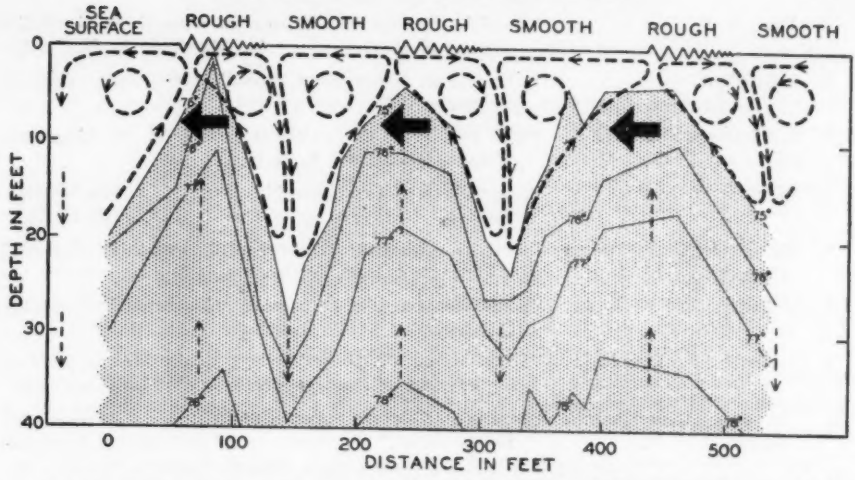


FIG. 30. Horizontal temperature section (fine lines), showing shallow internal waves which move from right to left (broad arrows), the probable turbulent (rotary) circulation (dashed arrows); and the rough and smooth bands at the surface (see Fig. 12 I).

the many unsolved problems must be a cooperating-team approach, whereby experts in one field work jointly with experts in another. This has been accomplished at Andhra University, as evidenced by the progress made on the many problems.

This summary also points out how interesting and thought-provoking the problems of the sea can be. The Bay of Bengal is literally a gold mine of intriguing problems for an unlimited number of future students in oceanography.

ACKNOWLEDGMENTS

The writer is grateful to the Heads of the three Departments, Professors C. Mahadevan, S. R. Savur and P. N. Ganapati, for continued friendly co-operation, and to the students of oceanography at Andhra University for their work at sea and in the laboratory, which is summarized here. Thanks is also extended to Dr. J. N. Nanda for his helpful interest in the work, especially for obtaining ship facilities and reviewing reports, and finally to Sir C. V. Raman who suggested the preparation of this report.

REFERENCES

1. Sewell, R. B. Seymour .. "The oceans around India. An Outline of Field Science in India," *Indian Science Congress*, 1937, 17–41.

2. Anonymous .. "Flood damage and flood control activities in Asia and the far east," *Flood Control Series No.* 1, Bureau of Flood Control, Economic Commission for Asia and the Far East, United Nations, Bangkok, 1950.

3. Dietz, R. S. .. "Possible deep-sea turbidity-current channels in the Indian Ocean," *Bull. Geol. Soc. An.*, March 1953, **64**, 375–76.

4. Kiilerich, A. .. "The Ganges Submarine Canyon," *Andhra Univ. Mem. in Oceanog.*, 1957, **2** (in press).

5. Kukkuteswara Rao and La Fond, E. C., "The profile of the continental shelf off Visakhapatnam Coast," *ibid.*, 1954, **1**, 78–85.

6. Sewell, R. B. Seymour .. "Geographic and Oceanographic research in Indian Waters," *Mem. of Asiatic Society of Bengal*, 1925, **9** (2), 27–50.

7. Mahadevan, C. and Poornachandra Rao, M. "Study of ocean floor sediments off the east Coast of India," *Andhra Univ. Mem. in Oceanog.*, 1954, **1**, 1–35.

8. Mahadevan, C. .. "Some recent results on oceanographic research at Andhra University," 1955 (in Press).

9. Poornachandra Rao, M. .. "Some aspects of marine geology in the Waltair parts of Bay of Bengal," *Doctorate Thesis*, Andhra University, 1954.

10. Mahadevan, C. and Poornachandra Rao, M. "Studies on the ocean bottom samples from the Bay of Bengal Indian Ocean," *Proceedings of XX International Geologica-Congress*, Mexico City, 1956 (in Press).

11. Poornachandra Rao, M. and Mahadevan, C. "The collections on the continental shelf off the Visakhapatnam area. A.I.O.P.," *Proc. Verb.*, 1955, No. 6, 292–93.

12. Mahadevan, C. and Aswathanarayana, U. "Radioactivity of sea floor sediments off the east coast of India," *Andhra Univ. Mem. in Oceanog.*, 1954, **1**, 36–50.

13. Borreswara Rao, C. and LaFond, E. C. "Sand sorting on the east coast beaches," *Curr. Sci.*, 1956, **25**, 77–78.

14. Rao, B. S. R. and Bhimasankaram, V. L. S. "Magnet metrical tracing of the heavy mineral concentrates of the Visakhapatnam Beach," *Indian Minerals*, 1956, **9** (4), 305–08.

15. LaFond, E. C. and Borreswara Rao, C. "Deposition and sorting of heavy mineral sands on the east coast beaches of India," *Proceedings of XX International Geological Congress*, Mexico City, 1956 (in Press).

16. ———— and Prasada Rao, R. "On the erosion of the beach at Uppada," *The Port Engineer*, 1956, **5** (2), 2–9.

17. ———— .. "Beach erosion cycles near Waltair on the Bay of Bengal," *Andhra Univ. Mem. in Oceanog.*, 1954, **1**, 63–77.

18. Prasada Rao, R. .. "Further studies of sand movement across Waltair Beach," *Curr. Sci.*, 1954, **23**, 48–49.

19. LaFond, E. C. and Prasada Rao, R. "Beach erosion cycles near Waltair on the Bay of Bengal," *Andhra Univ. Mem. in Oceanog.*, 1954, **1**, 63–77.

20. ———— "On the swatch and backwash," *Oceanography of Bay of Bengal, Central Board of Physics*, May 1956, pp. 18–19.

21. Bhavanarayana, P. .. "Foam on the sea," *ibid.*, Calcutta, May 1956, p. 17.

22. Satyanarayana Rao, T. S... "Studies of the penetration of light in the Bay of Bengal, Part 1. Transparency of the waters on the east coast of India and its significance," 1957 (in Press).

23. LaFond, E. C. and Sastry, J. S. "Turbidity of waters off the east coast of India," *Indian Jour. of Met. and Geoph.* (in Press).

24. Sewell, R. B. Seymour .. *Memoirs of the Asiatic Society of Bengal*, 1929, **9** (5 and 6), 207–423.

25. LaFond, E. C. .. "Seasonal cycle of sea surface temperature and salinity along the east coast of India," *Andhra Univ. Mem. in Oceanog.*, **2** (in Press).

26. Ganapati, P. N. and Murty, V. S. R. "Salinity and temperature variations of the surface waters off the Visakhapatnam coast," *ibid.*, 1954, **1**, 125–41.

27. LaFond, E. C. .. "On upwelling and sinking off the east coast of India," *ibid.*, 1954, **1**, 117–21.

28. Ganapati, P. N., LaFond, E. C. and Bhavanarayana, P. V. "On the vertical distribution of chemical constituents in the shelf waters off Waltair," *Proc. Ind. Acad. Sci.*, 1956, **44 B** (2), 68–72.

29. Bhavanarayana, P. V. and LaFond, E. C. "On the replenishment of some plant nutrients during the upwelling period on the east coast off India," *Indian Jour. Fish.*, 1957, **4** (1), 75–79.

30. Ganapati, P. N. and Satyanarayana Rao, T. S. "Distribution of the Chætognatha in the Waters off Visakhapatnam Coast," *Andhra Univ. Mem. in Oceanog.*, 1954, **1**, 143–50.

31. ——— and Rama Mohan Rao, V. "Studies of plankton copepods. 1. Seasonal fluctuations in the distribution with reference to salinity and temperature," *ibid.*, 1954, **1**, 151–62.

32. Sastry, A. N. .. "The littoral bottom fauna on the Coromandal Coast," *Oceanography of Bay of Bengal, Central Board of Geophysics*, Calcutta, May 1956, p. 25.

33. Ganapati, P. N., Lakshmana Rao, M. V. and Nagabhushanam, R. "A preliminary account of the fouling organisms of the Visakhapatnam Harbour area" (in Press).

34. LaFond, E. C. .. "On upwelling and fisheries," *Curr. Sci.*, August 1955, **24**, 258–59.

35. Panikkar, N. K. .. "Annual Report of the Chief Research Officer, 1953," *Indian Jour. Fish.*, 1953, 377.

36. Ganapati, P. N. and Satyanarayana Rao, T. S. "The scope of fisheries in the Bay of Bengal," *Indian Science Congress Abst.*, 1956.

37. Anonymous .. *Indian Ocean, Oceanographic and Meteorological Data*, Koninklijk Nederlands Meteorologisch Institut No. 135, 2nd Ed., 1952.

38. LaFond, E. C. .. "Sea surface density at four stations along the east coast," *Indian Jour. of Met. and Geoph.* (in Press).

39. ——— .. "Environmental factors affecting the vertical temperature structure of the upper layers of the sea," *Andhra Univ. Mem. in Oceanog.*, 1954, **1**, 94–101.

40. ——— .. "Bathythermograms—an oceanographic tool," *Curr. Sci.*, 1956, **25** (9), 40–41.

41. ——— and Borreswara Rao, C. "Vertical temperature structure of the upper layers of the sea off the east coast of India," *Defence Science Organization Publication No.* 4/55, 1955, 1–89.

42. LaFond, E. C. .. "On the circulation of the surface layers off the east coast of India," *Andhra Univ. Mem. in Oceanog.*, **2** (in Press).

43. Poornachandra Rao, C. .. "Meteorological factors affecting sea temperature and salinity off Waltair," *Curr. Sci.* (in Press).

44. LaFond, E. C. and Bhavanarayana, P. V. "On a thermal feature of bottom water during upwelling on the east coast of India," *Indian Jour. of Met. and Geoph.*, 1956 (in Press).

45. Prasada Rao, R. and LaFond, E. C. "Changes in sea-level at Visakhapatnam on the east coast of India," *Andhra Univ. Mem. in Oceanog.*, 1954, **1**, 86–93.

46. LaFond, E. C. and Poornachandra Rao, C. "Vertical oscillations of tidal periods in the temperature structure of the sea," *ibid.*, 1954, **1**, 109–16.

47. ——— and Borreswara Rao, C. "Rotary currents in the Bay of Bengal," *ibid.*, 1954, **1**, 102–08.

FIG. 2. Oceanographic equipment and measurements. (A) I.N.S. Rohilkhand used for oceanographic work. (B) Level-winding wire rope on electric winch drum. (C) Retrieving Phleger corer. (D) Extracting sea floor sediment core from corer. (E) Examining marine samples. (F) Retrieving sea floor sediment snapper. (G) Extracting sediment from snapper. (H) Placing glass slide in bathythermograph for temparture. (I) Lowering bathythermograph off stern of ship.

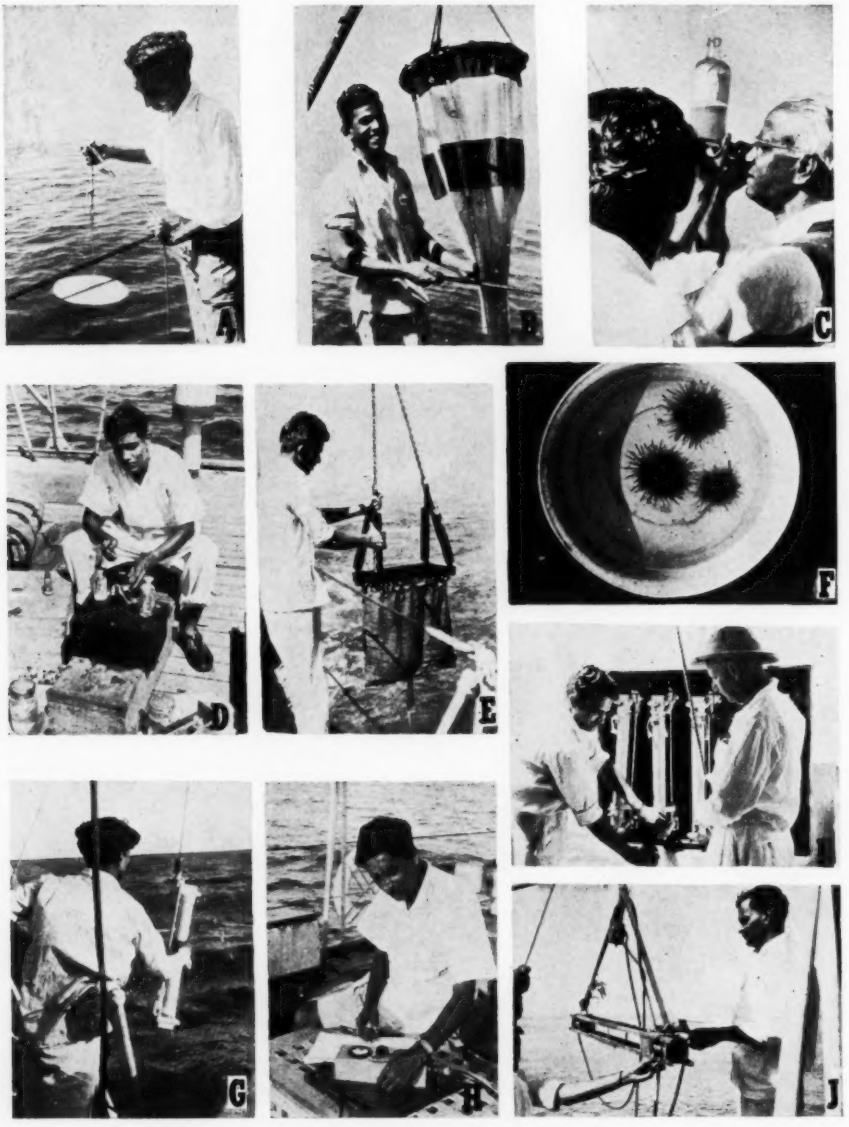


FIG. 3. Oceanographic equipment and measurements. (A) Lowering Secchi disc for determination of transparency of water. (B) Lowering plankton net to collect small plants and animals. (C) Examining plankton collection. (D) Making chemical analysis of sea-water. (E) Lowering dredge to scoop up benthonic organisms. (F) New sea urchin—*Diadema* sp.—dredged off Kalingapatam. (G) Retrieving Nansen bottle with water sample. (H) Reading hydrophotometer meters on deck. (I) Drawing water samples from Nansen bottles. (J) Lowering hydrophotometer to measure water transparency.

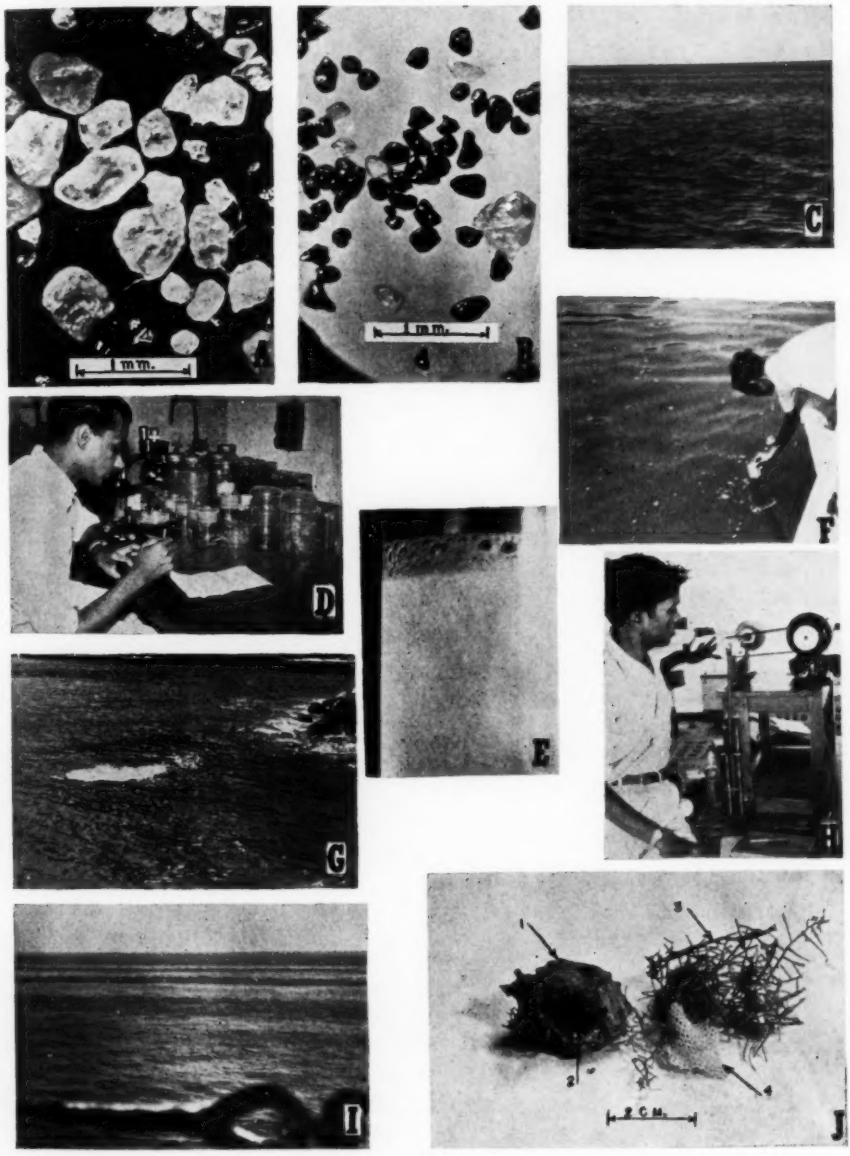


FIG. 12. Oceanographic samples, equipment and procedures. (A) Sample of normal beach sands. (B) Sample of heavy mineral beach sands. (C) Sea surface slicks. (D) Examining biological samples in Laboratory. (E) Foam layer produced by shaker. (F) Sampling film from sea surface slick. (G) Persistent foam patch on sea. (H) Shaking samples of water for foam study. (I) Surface appearance of shallow internal waves (rotary currents). (J) Dredge sample (sta. 704, depth 35 fms.). (1) Sponge, (2) Pecten, (3) Hydroid, (4) Bryozoa.

IONIC CHANGES IN UNSTRIATED MUSCLE IMMERSED IN SODIUM-FREE SOLUTIONS

By Inderjit Singh, F.A.Sc. and A. K. Acharya

(From the Department of Physiology, Medical College, Agra)

Received May 16, 1957

Singh and Singh (1943), Singh (1944, 1957) and Singh and Bhatt (1957 *a*, *b*), found that the stomach muscle of the frog, *Rana tigrina*, had the remarkable property, that it remained irritable in hypotonic (0·112 *M*) solution of sucrose for over 24 hours. They found that the muscle at first acclimatises to sucrose solution, and the contractions in sucrose are several times bigger than in saline and are accompanied by action potentials. When the muscle has acclimatised to sucrose, introduction of sodium chloride suppresses the spontaneous contractions till the muscle has acclimatised again to sodium. This shows that the spontaneous contractions could not be due to the retention of sodium in the interspaces between the fibres. This muscle has another remarkable property that it remains irritable in sodium-free solutions of other ions, such as ammonium, potassium, calcium, strontium and magnesium chlorides.

In the present research these experiments have been extended and the sodium and potassium content of the muscle and experimental solutions have been determined under various experimental conditions.

Material and Methods

Transverse pieces of the stomach muscle of the frog, *Rana tigrina*, were used. They were suspended in saline (Singh, 1939) in the muscle chamber, and then contractions recorded. The saline was then replaced with hypotonic sucrose solution (0·112 *M*), or other sodium-free solutions. These were renewed every 15 minutes. At the end of 4 hours, when the spontaneous contractions were several times bigger than in saline, the muscle was removed for analysis.

The muscle was gently blotted between ash-free filter-papers and weighed. It was dried by heating at 105° C. for 24 hours. It was then digested in 50 per cent. nitric acid as described by McDowall, Munro and Zayat (1955). Sodium was estimated by EEL's flame photometer.

These experiments were performed at room temperature, 30° C.

Results

Sodium in the sucrose solution.—Even the purest sucrose (AR, B.D.H.) contains traces of sodium but no potassium, as shown by the flame photo-

meter; the colour of the flame is no more yellow, but still a slight deflection of the galvanometer occurs when tested for sodium. The hypotonic solution of sucrose, 0·112 *M*, used in these experiments, contained 0·0035 m.Eq. of sodium per litre. That this trace of sodium in the external medium is of no importance for excitability, is shown by the fact that in the summer, the heart and the sartorius muscle from the same animal loose their excitability completely within 10 minutes of immersion in the sucrose solution. In the winter season, they last longer, remaining excitable for about an hour. The frog's stomach muscle, however, behaves better in summer than in winter.

When the muscle is immersed in sucrose solution, sodium begins to diffuse out. The outward diffusion of sodium from the muscle into the sucrose solution was tested by immersing the muscle in 100 ml. of oxygenated solution and analysing the solution at the end of every 15 minutes, the sucrose solution being renewed each time. At the end of 2½ hours, very little sodium comes out of the muscle, and at this time the spontaneous contractions begin (Table I). Thus when the muscle has acclimatised to sucrose, the more the sodium is removed from the interspaces, the better it contracts. The contractions in the sucrose solution, therefore, could not be due to retention of any sodium in the interspaces.

TABLE I

Frog's stomach muscle. The amount of sodium leaking out of the muscle into 100 *ml. of nypotonic*, 0·112 M *sucrose*

Time of immersion in sucrose (minutes)	m.Eq. of sodium per litre of sucrose solution
0	112
15	22·2
30	4·7
45	1·2
60	0·8
90	0·4
120	0·08
150	0·0035
180	0·0035

The above conclusion is also substantiated by the fact, that re-immersion of the muscle in sodium chloride solution, completely suppresses the spontaneous contractions. This also happens, if the sodium chloride contains a little calcium chloride, normally present in the saline (Fig. 1). Suppression

of the spontaneous contractions is still produced if the sodium chloride solution besides containing calcium, also contains a little potassium normally present in the saline.

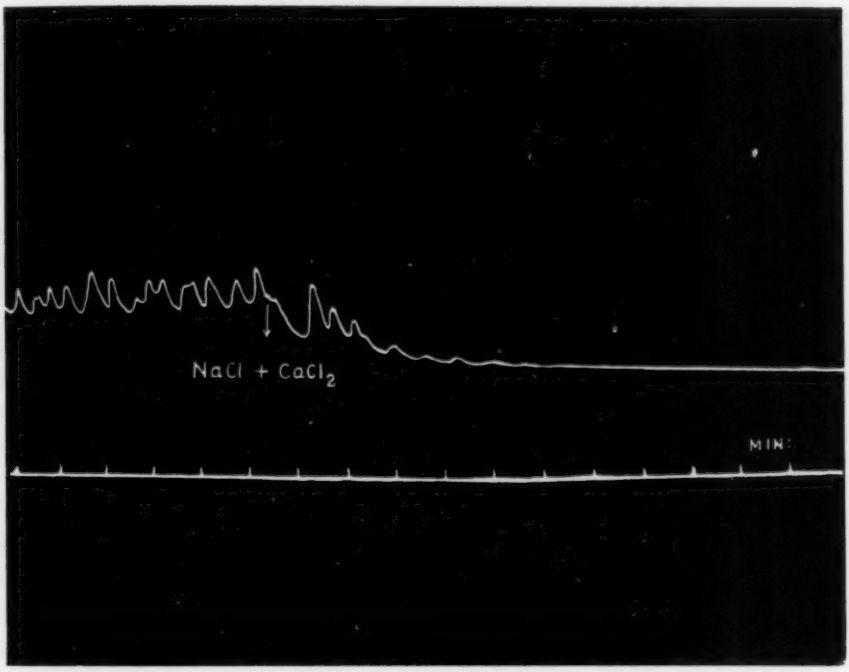


FIG. 1. Frog's stomach muscle. The muscle is contracting rhythmically in hypotonic (0·112 *M*) sucrose. Immersion in 0·108 *M* sodium chloride with 0·00412 *M* calcium chloride produces immediate depression of tone and the spontaneous contractions.

Sodium and potassium in the muscle.—The sodium and potassium content of 12 muscles immersed in hypotonic sucrose, 0·112 *M*, are shown in Table II. The mean sodium concentration in the muscle is 0·0018 m.Eq. per ml. of water in the muscle. The mean potassium concentration is 0·03 m.Eq. The total sodium and potassium is equal to 0·0318 m.Eq. per ml.

The potassium content of unsoaked muscle is about 0·06 m.Eq. per gram of wet muscle (Gokhale and Singh, 1945). The potassium content of muscle, soaked in sucrose solution, is therefore, about half this amount, owing to the hypotonicity of the solution.

The mechanical response of the muscle gradually declines in sucrose solution. This is presumably due to leakage of potassium. The sodium

TABLE II

Sodium and potassium content of frog's stomach muscle immersed in 0·112 M *sucrose solution for* 4 *hours, exhibiting vigorous spontaneous contractions; these are intracellular ions*

Serial No.	m.Eq.			
	Na		K	
	Per gram of wet muscle	Per ml. of muscle water	Per gram of wet muscle	Per ml. of muscle water
1	0·0015	0·0017	0·028	0·033
2	0·0015	0·0008	0·025	0·030
3	0·0019	0·0024	0·025	0·031
4	0·0019	0·0025	0·026	0·035
5	0·0010	0·0012	0·027	0·033
6	0·0010	0·0012	0·024	0·036
7	0·0020	0·0024	0·028	0·034
8	0·0015	0·0016	0·017	0·020
9	0·0017	0·0020	0·020	0·020
10	0·0015	0·0019	0·020	0·025
11	0·0012	0·0015	0·022	0·027
12	0·0019	0·0021	0·029	0·032
Mean ..	0·0016	0·0018	0·024	0·030
S.D. ..	± 0·0003	± 0·0005	± 0·003	± 0·005

TABLE III

Sodium and potassium content of frog's stomach muscle immersed in 0·112 M *sucrose solution at different intervals; these are intracellular ions*

Sl. No.	m.Eq. per gram of wet muscle					
	After 1 hour		After 5 hours		After 9 hours	
	Na	K	Na	K	Na	K
1	0·0045	0·038	0·0017	0·021	0·0012	0·016
2	0·0034	0·022	0·0016	0·010	0·0013	0·008
3	0·0030	0·036	0·0023	0·030	0·0017	0·020
Mean ..	0·0036	0·032	0·0019	0·020	0·0014	0·015
S.D. ..	± 0·0006	± 0·007	± 0·0004	± 0·008	± 0·0003	± 0·005

and potassium contents of the muscle soaked in various periods in the hypotonic sucrose solution are shown in Table III.

Discussion

These experiments show that it is the intracellular potassium rather than the extracellular sodium that is mainly responsible for excitability. Various other experiments point to the same conclusion. Thus the phasic response of the muscle is related to its intracellular potassium. It increases with increase of the osmotic pressure of the medium to 1·4 times normal (Singh) 1939), which increases the intracellular concentration of potassium by about 35 p.c. (Gokhale and Singh, 1945). The muscle is excited if the osmotic pressure of the medium is increased suddenly to 1·4 times normal (Singh, 1942). Similarly the muscle is excited by relative increase in the intracellular concentration of ions caused by withdrawal of ions from the medium by immersing the muscle in sucrose solution. The muscle is also excited if there is absolute or relative increase in the concentration of extracellular ions. Thus excess of potassium outside causes contraction.

An interesting feature is that the intracellular potassium can be partly replaced with ammonium (Gokhale and Singh, 1945), and if after immersion in saline containing ammonium, the muscle is re-immersed in ammonium-free saline, a contraction is caused (Singh, 1939). Thus a difference in concentration of ammonium on two sides of the muscle membrane stimulates the muscle.

Tonic contraction of the muscle is presumably due to the action of intracellular ions on the actomyosin system. Increase in the intracellular concentration of potassium increases the phasic response as well as one kind of tone, but decreases another kind of tone (Singh, 1939). This suggests that in the muscle, there are two systems of polypeptide chains, one responsible for the phasic and one kind of tonic response, and the other for the second kind of tonic response. These two systems can be differentially destroyed; thus sudden stretching destroys one kind of phasic response (Singh and Singh, 1951 *a*, Singh, 1952) or the second kind of tonic response (Singh and Singh, 1949, 1950 *a*). One of these systems relaxes actively, and the other passively (Singh and Singh, 1950 *b*, 1951 *b*).

The frog's stomach muscle, immersed in sucrose solution, can be stimulated if the electrodes are placed at a distance of about 1–2 inches on either side of the muscle. The intervening solution is practically non-conducting, so that it might appear that the contractile system of the muscle is stimulated by the longitudinal electrical field (Singh, 1944; Singh and Bhatt, 1957 *b*). It is tempting to assume that this experiment provides the missing link between

the excitability and the contractile systems of the muscle and supports the "Window field" theory (Szent Gyorgyi, 1953). This would be if the muscle responds to electric current only. But the muscle contracts spontaneously, and reacts to adrenaline and acetylcholine. In fact, it behaves in every way like a normal muscle, though, there are no ions in the external medium. This shows that its excitatory system is functioning normally. The muscle is undoubtedly stimulated by the longitudinal electrical field (0·8 cm./volt), but the action is on the membrane and not on the contractile mechanism.

The frog's stomach muscle also responds if the sodium of the saline is partly or completely replaced with potassium (Singh and Singh, 1947; Singh and Bhatt, 1957); this abolishes the sodium and potassium concentration gradients across the muscle membrane. It would again be tempting to assume that the contractile system of the muscle is stimulated by the longitudinal electrical field, if it responded only to electrical current. But since it also responds to acetylcholine, we presume that the electrical field acts on the membrane and not on the contractile mechanism; we are, therefore, unable to agree with Csapo (1954).

SUMMARY AND CONCLUSIONS

1. Frog's stomach muscle responds when immersed in hypotonic sucrose solution. The more thoroughly, sodium is eliminated from the external medium, the better it responds.

2. After the muscle has acclimatised to sucrose solution, re-immersion in solution of sodium chloride, or sodium chloride containing a little calcium or potassium abolishes the spontaneous contractions.

3. The sodium and potassium concentration in the muscle after immersion in sucrose for 4 hours becomes 0·0018 m.Eq. and 0·03 m.Eq. respectively per ml. of water in the muscle.

4. The mechanical response of the muscle in sucrose solution varies as the intracellular potassium.

5. These experiments suggest that intracellular potassium, and not extracellular sodium, that is mainly responsible for excitability of the muscle.

REFERENCES

Csapo, A. .. *Nature*, 1954, **173**, 1019.

Gokhale, S. K. and Singh, I. .. *Proc. Ind. Acad. Sci.*, 1945, **21**, 202.

McDowall, R. J. S., Munro, A. F. and Zayat, A. F. *J. Physiol.*, 1955, **130**, 615.

Singh, I. .. *J. Physiol.*, 1939, **96**, 367.

———— .. *Ind. Journ. Med. Res.*, 1942, **30**, 629.

———— .. *Curr. Sci.*, 1944, **10**, 251.

———— .. *Ibid.*, 1952, **21**, 17.

———— .. *Presidential Address, Physiology Section, Indian Science Congress, 44th Session, Calcutta*, 1957.

——— and Bhatt, J. V. .. *Curr. Sci.*, 1957 *a*, **26**, 17.

———— .. *Proc. Ind. Acad. Sci.*, 1957 *b*, **45**, 64.

Singh, S. I. and Singh, I. .. *Ibid.*, 1943, **18**, 58.

———— .. *Ibid.*, 1947, **26**, 211.

———— .. *Ibid.*, 1949, **30**, 263.

———— .. *Ibid.*, 1950 *a*, **31**, 351.

———— .. *Nature*, 1950 *b*, **166**, 647.

———— .. *Curr. Sci.*, 1951 *a*, **20**, 130.

———— .. *Nature*, 1951 *b*, **167**, 564.

Szent Gyorgyi, A. .. *Chemical Physiology of Contraction in Body and Heart Muscle*, 1953, Academic Press Inc., New York, pp. 117.

ELECTRON MICROSCOPY AND ALGOLOGY*

By T. V. Desikachary, F.A.Sc.‡
(*University of Saugar, Sagar, M.P.*)

Received May 3, 1957

In 1936 the first alga, a diatom, was photographed with the electron microscope, by Krause. Since then more and more algæ from the Chlorophyceæ, Chrysophyceæ, Bacillariophyceæ, Cyanophyceæ and Phæophyceæ have been studied using this powerful tool. Among the specific aspects under intensive study may be mentioned wall structure, flagellar structure and the structure of the chloroplasts, chromatophores and pyrenoids. These investigations with the aid of the electron microscope (hereafter cited as EM) have already contributed a great deal on the micromorphology of algæ. In many cases they have supplemented observations with the optical microscope while in a few cases they have supplanted the latter. Some of the important contributions in this new and fascinating field of research are reviewed here with a view to understand the full import of these investigations into the micromorphology of algæ.

Wall Structure

Bacillariophyceæ.—Kolbe and Gölz (1943), Gerloff and Gölz (1944), Hustedt (1945, 1952), Kolbe (1948, 1951, 1954), Helmcke and Krieger (1951 *a*, 1951 *b*, 1951 *c*, 1951 *d*, 1952 *a*, 1952 *b*, 1952 *c*, 1952 *d*, 1953, 1954 *a*, 1954 *b*), Okuno (1944 *a*, 1944 *b*, 1947, 1948 *a*, 1948 *b*, 1949 *a*, 1949 *b*, 1950 *a*, 1950 *b*, 1951, 1952 *a*, 1952 *b*, 1952 *c*, 1953 *a*, 1953 *b*, 1953 *c*, 1953 *d*, 1954 *a*, 1954 *b*, 1954 *c*, 1954 *d*, 1954 *e*, 1954 *f*, 1955 *a*, 1955 *b*), Desikachary (1952, 1954 *a*, 1954 *b*, 1954 *c*, 1956), Desikachary and Aleem (1955), and Desikachary and Kanwar Bahadur (1954 *a*, 1954 *b*, 1954 *c*) have studied a large number of diatoms for the structure of the areolæ, striæ, spines, etc. Hustedt (1945, 1955), Kolbe (1948), Okuno (1953 *c*) and Desikachary (1954 *c*) have summarised the results of these different studies and their bearing on the classification of the areolæ. Kolbe (1951), Helmcke and Krieger (1952 *b*, 1953, 1954 *a*) and, Fott and Rozsival (1950) have described the canal raphe of Nitzschiaceæ and Surirellaceæ. A full review of these investigations has been published by the present writer (1956).

---

* Modified version of paper read at the First Regional Conference on "Electron Microscopy, in Asia and Oceania," held at Tokyo in October 1956.

‡ Now at the University Botany Laboratory, Madras-5.

Chrysophyceæ.—The structure of the scales in Chrysophyceæ (Manton, 1955; Parke, Manton and Clarke, 1954, 1956; Asmund, 1955; Houwink, 1951, Petersen and Hansen, 1956) and in particular of the coccoliths (Braarud, 1954 *a* and 1954 *b*; Braarud and Nordli, 1952; Braarud, Gaarder, Markali and Nordli, 1952; Gaarder, Markali & Ramsfjell, 1954; Gaarder & Markali, 1955 *a*; Halldal and Markali, 1954 *a*, 1954 *b*; Deflandre and Fert, 1952; Downie and Honeycomb, 1956) have been, probably for the first time, described in very great detail with the help of the EM. Based on these EM studies Braarud, Deflandre, Halldal and Kamptner (1955) give a new classification of the coccoliths. They distinguish three main types of coccoliths: I. Holococcoliths, *e.g.*, crystalloliths from *Crystallolithus hyalinus*, calyptroliths from *Sphærocalyptra papillifera* and zygoliths from *Homozygosphæra triarch*, II. Heterococcoliths, *e.g.*, cricoliths from *Hymenomonas carteræ*, cyrtoliths from *Anthosphæra* and *Discosphæra tubifer*, caneoliths from *Syracosphæra mediterranea*, placoliths from *Coccolithus huxleyi* and scapholiths from *Anoplosolenis brasiliensis*, and III. Pentaliths, *e.g.*, *Braarudosphæra bigelowi*. The same classification was further enlarged by Halldal and Markali (1955) who also give a full list of coccoliths studied so far. These two authors recognise in addition to the above two more types of coccoliths, *viz.*, Lepidoliths from *Thorosphæra flabellata* and Cribriliths from *Pontosphæra discopora*.

Chlorophyceæ.—The fibrillar structure of the wall, their lamellation and their relation to the inner protoplasmic contents as well as their chemical composition were studied by Preston and Nicolai and their collaborators (Preston and Kuyper, 1951; Preston *et al.*, 1953, 1948; see also Preston, 1954 especially, pp. 91–112) in a number of green algæ belong to the cœnocytic orders. These investigators have observed evidences which have some taxonomic significance. In *Pediastrum* spp. Moner (1955) has observed the cell-wall to be made up of two layers, a continuous internal membrane and a hexagonally reticulate layer on the outside.

Cyanophyceæ.—The structure of the sheath in the blue-green algæ has been studied by Bringmann (1951), Frey-Wyssling (1954) and Singh (1954). According to Singh the sheath in *Scytonema pseudoguayanense* consists of a two directional arrangement of cellulose fibrils in a menstrum of proteinaceous material.

A dinoflagellate, *Exuviella baltica*, was studied recently by Braarud, Markali and Nordli (1955; see Braarud, 1955) and they have observed the presence of small spines on the wall.

FLAGELLAR STRUCTURE

The studies of Manton and her collaborators (Manton, 1952, 1953, 1955; Manton and Clarke, 1951 *a* and *b*; Manton *et al.*, 1952) on the structure of the flagella in some flagellates and also in the reproductive bodies of certain algæ are classical examples illustrating the limits of the optical microscope and the potentialities of the EM in the ultramicroscopic studies of algæ. The structure of the flagellum as described by them (see Manton, Clarke and Greenwood, 1953, Text-Figs. 1–3) with 9 peripheral fibrils and two axial fibrils, probably, is the basic pattern of the structure of the flagellum in all the flagellates with variations (see Manton *et al.*, 1953, p. 328). The structure of the flagellum in *Synura* was studied by Manton (1955) and in six species of *Chrysochromulina* by Parke, Manton and Clarke (1954, 1956). The latter authors have also described the structure of what was for a long time known as the third flagellum, ' Haptonema ', and have shown clearly how far the observations made with the optical microscope had gone wrong. Lewin and Meinhart (1953) have described in great detail the role of the flagella in mating in *Chlamydomonas mœwusii* and the formation of a protoplasmic bridge between copulants during mating. Petersen and Hansen (1954) have described the collar in *Codonosigia botrytis* as a ring of very fine protoplasmic tentacles or threads. Among the many other studies on flagella may be mentioned also those of Chen (1949), Houwink (1951), Brown (1951) and Astbury and Saha (1953).

CHLOROPLASTS AND PYRENOIDS

Chloroplasts or chromatophores and pyrenoids have been studied in a few algæ such as *Spirogyra*, *Euglena*, *Enteromorpha intestinalis*, *Cladophora glomerata*, *Mougeotia* sp., *Closterium acerosum* and *C. lunula* (Steinmann, 1951; Wolken and Palade, 1952, 1953; Leyon, 1954). According to Leyon the pyrenoids occur in chloroplasts which do not have the well-known ' grana '. The pyrenoids themselves, are lamellated, the lamellæ being three to four times as thick as and continuous with the lamellæ of the chloroplast. The arrangement of the lamellæ in the pyrenoids is varying in different algæ. The work of Leyon has to be followed up by investigations on diverse other algæ, especially the chloroplasts of such related algæ as *Ulothrix* and *Microspora* with or without pyrenoids, and if proved to be true of many others too, a change in the concept of the structure of the pyrenoids, etc., as is now known from the work on light microscope (see Fritsch, 1935, 65) would seem necessary.

Electron Microscopy and Taxonomy

The impact of these investigations on the microstructure of algæ has been pointed above. There is however one point which is being much debated at present, *i.e.*, the utilization of the microstructure as observed with the EM in taxonomy, and indeed taxonomists have expressed divergent opinions on the subject as is apparent from selected literature referred to below.

Kolbe (1948, 1951) and Helmcke and Krieger (1954 *a*) have effected taxonomic changes based on evidences from EM studies. Desikachary (1952 and 1956) envisages their utilization in taxonomy and suggests that identification be based on gross or major characters assessed by the light microscope and confirmed by studies of the minute characters with EM. However, Hustedt (1952, 1955) doubts their taxonomic value. It seems hardly necessary to emphasize that a right understanding of the details of the areolar structure will lead to a correct and precise concept of genera and species and in any event be a check on the creation of new and doubtful taxa on meagre evidences. The real identity of *Nitzschia closterium* f. *minutissimum* (see Hendey, 1954) is a revelation made possible only by the EM. A large amount of our present knowledge of the physiology of the diatom is based on this organism and is in need of revision.

The taxonomy of coccolithophorids is based on the structure of the coccoliths. On account of their very minute size it has not been possible to have a satisfactory and complete picture of their structure, even with the most advance optical equipment. This handicap has been cause for a great amount of confusion in their classification. Braarud and Nordli (1952) have shown by EM studies of the coccoliths that *Pontosphæra huxleyii* includes three distinct species one of *Gephyrocapsa* and two of *Coccolithus* and none of *Pontosphæra* itself. In other cases too the coccoliths have been found to be different from that known from studies with the light microscope. For example, *Syracosphæra carteræ* Braarud et Fagerland has been known to have discoliths (Schiller, 1930) but has been shown to have tremaliths type of coccolith by EM studies. Braarud (1954) based on a study of *Hymenomonas* found a similarity in structure of the coccoliths of *Hymenomonas roseola* and *Syracosphæra cartaræ* and transferred the latter to the former genus as *H. carteræ*. Braarud *et al.* (1952) are of the view that further studies in the EM of other coccolithophorids may reveal many details of the coccolith morphology that a new coccolith classification may be necessary and possibly also a reclassification of the Coccolithophoridaceæ (see also Deflandre and Fert, 1952 and Braarud *et al.*, 1955). Braarud (1954 *a*)

feels that many other taxonomic questions within the group may have to be reconsidered when further studies have given a more satisfactory basis for evaluating the various characters for a taxonomic distinction between the species and genera of coccolithophorids. Halldal and Markali (1954 *b*) have recognised the importance of these studies. " During the short period electron microscopy has been used in the investigation of coccolithophorids it has become apparent that the taxonomy of the group needs to be reviewed on several points. However, far more observations are needed to obtain satisfactory rearrangement of the species." It has seemed to these authors (1954 *a*) especially worthwhile to study in the EM coccoliths of cells which are first examined in the light microscope and identified according to present-day monographs.

Halldal and Markali (1955, p. 20) write that the inclusion of Electron micrographs of coccoliths in the classification appear to them to be mandatory.

Parke *et al.*, (1954, 1956) in their studies with *Chrysochromulina* spp., observed certain characters which introduced some doubt in the specific identification by the light microscope. But they refrain from establishing genera and species on characters which cannot be seen with the light microscope.

Petersen and Hansen (1956), in their studies on *Synura* spp., say that in future it will be necessary to make use of electron micrographs of scales in order to determine the species with adequate precision.

Braarud (1955) in a recent paper has ably summarised the probable use of these studies in the taxonomy of marine plankton.

Thus it may be seen from the above that opinions of taxonomists in the matter of utilization of the results of EM studies in micromorphology in different groups of algæ vary from scepticism to one of extreme enthusiasm for the new structural knowledge gathered by using this powerful tool. There are still workers who would like to use these evidences with reservations. The present need is probably to first collect complete and extensive data based on studies with the EM of species and genera determined earlier with the help of light microscope. Only then we would be in a position to evaluate them to yield a reasonably workable synthesis of characters derived both by light and electron microscopic studies as may serve in taxonomy.

SUMMARY

Since 1936, when the first alga was investigated with the help of the Electron Microscope more and more algæ have been studied. These studies have greatly contributed to our knowledge of the submicroscopic morpho-

logy of algæ. A review of the most important aspects of these investigations is given in this paper.

The utilization of the results of these Electron Microscopic investigations in taxonomy is discussed.

References

Asmund, B. .. "Electron microscope observations on *Mallomonas caudata* and some remarks on its occurrence in four desmid ponds," *Bot. Tidsskr.*, 1955, **52,** 163–68.

Astbury, W. T. and Saha, N. N. "Structure of algal flagella," *Nature, Lond.*, 1953, **171,** 280–83.

Braarud, T. .. "Coccolith morphology and taxonomic position of *Hymenomonas roseola* Stein and *Syracosphæra carteræ* Braarud and Fagerland," *Nytt. Mag. Bot.*, 1954 *a*, **3,** 1–4.

———— .. "The study of plankton algæ in electron microscope," *Blyttia*, 1954 *b*, **2,** 102–08.

———— .. *Electron Microscopy in Oceanographic Research. Papers in Marine Biology and Oceanography* (Pergamon Press Ltd., London, 1955, 479–81).

——, Deflandre, G., Halldal, P. and Kamptner, E. "Terminology, nomenclature and systematics of Coccolithophoridæ," *Micropalæontology*, 1955, **1** (2), 157–59.

——, Gaarder, K., Markali, J. and Nordli, E. "Coccolithophorids studied in the electron microscope. Observations on *Coccolithus huxleyi* and *Syracosphæra carteræ*," *Nytt. Mag. Bot.*, 1952, **1,** 129–34.

——, Markali, J. and Nordli, E. "A note on the thecal structure of *Exuviella baltica* Lohm.," *Ibid.*, 1955, **4** (In Press, cited from Braarud, 1955).

—— and Nordli, E. .. "Coccoliths of *Coccolithus huxleyi* seen in an electron microscope," *Nature, Lond.*, 1952, **170,** 361–62.

Bringmann, G. .. "Über elektronenmikroskopischen Fein strukturen der Membranscheiden von Oscillatoren," *Z. wiss. Mikr.*, 1951, **60,** 83–84.

Brown, H. P. .. "Note on the flagellar structure of *Chilomonas*, cryptomonad flagella," *Proc. Amer. Soc. Protozool.*, 1951, **2,** 11.

Chen, Y. T. .. "The flagellar structure of some protista," *Proc. Conf. Electron Microscopy, Delf.*, 1949, 156.

Deflandre, G. and Fert, Ch. .. "Sur la structure fine des quelques coccolithes fossiles observés an microscope électronique. Significance morphogenetique et application a la systematique," *C.R. Acad. Sci., Paris*, 1952, **234,** 2100–02.

———— .. "Étude des Coccolithophoridés des veses actuelles an microscope électronique: Orientation des particulares elementaires de calcaire en rapport avec les notions d'Heliolithe et Ortholithæ," *Ibid.*, 1953 *a*, **236,** 328–30.

———— .. "Application du microscope électronique à l'étude des Coccolithophoridés," *Bull. Soc. Hist. nat. Toulouse*, 1953 *b*, **60,** 301–13.

———— .. "Observations sur les Coccolithophoridés actuels et fossiles observes an microscope électroniques," *Ann. Palæont.*, 1954, **40,** 115–36.

Desikachary, T. V. .. "Electron microscope study of the diatom wall structure," *J. Sci. industr. Res.*, 1952, **11 B** (11), 491–500.

———— .. "Electron microscope study of the diatom wall structure, III," *Amer. J. Bot.*, 1954 *a*, **41** (8), 616–19.

———— .. "Electron microscope study of the diatom wall structure, VI," *Mikroskopie*, 1954 *b*, **9** (5/6), 168–78.

———— .. "The structure of the areolæ in diatoms," *VIII e Congres Int. Bot. Paris, Rapp. et Comm.*, 1954 *c*, Sect. **17**, 125–26.

———— .. "Electron microscope studies of diatoms," *J. R. micr. Soc.*, 1956, **76**, 9–36.

—— and Aleem, A. A. .. "Electron microscope study of diatom wall structure, VII," *J. Sci. industr. Res.*, 1955, **14 C** (2), 42–46.

—— and Kanwar Bahadur .. "Electron microscope study of diatom wall structure, II," *Ibid.*, 1954 *a*, **13 B** (2), 92–94.

———— .. "Electron microscope study of diatom wall structure, IV," *Ibid.*, 1954 *b*, **13 B** (4), 240–43.

———— .. "Electron microscope study of diatom wall structure, V," *Trans. Amer. Micr. Soc.*, 1954 *c*, **73** (3), 274–77.

Downie, C. and Honeycombe, R. W. "Examination of fossil coccoliths in the electron microscope," *Nature, Lond.*, 1956, **177**, 947–48.

Fott, B. and Rozsival, M. .. "Frustules of *Attheya zachariasii* in Electron Microscope," *Studia bot. cechosl.*, 1950, **11**, 262–67.

Gaarder, K. R. and Markali, J. "Morphological observations with the electron microscope on *Braarudosphæra bigelowi* (Gran and Braarud) Defil.," *Nytt. Mag. Bot.*, 1955 *a*, **4**.

———— .. *Crystallolithus hyalinus* n. gen., et sp. nov. (in Press), 1955 *b*.

———— .. "Observations with the electron microscope in *Halopappus adriaticus* (in Press), 1955 *c*.

Gaarder, K. R., Markali, J. and Ramsfjell, E. "Further observations on the Coccolithophorid from *Calciopappus caudatus*," *Avh. norske Videnskakad.*, 1954, 1–9.

—— and Ramsfjell, E. .. "A new Coccolithophorid from northern waters *Calciopappus caudatus* n. gen. et sp. nov.," *Nytt. Mag. Bot.*, 1954, **2**, 155–56.

Gerloff, I. and Gölz, E. .. "Über d. Feinbau d. Kieselschalen bei einigen Zentrischen Diatomeen," *Hedwigia*, 1944, **81**, 283–97.

Halldal, P. .. "Comparative observations on Coccolithophorids in light and electron microscope and their taxonomical significance," *Rapp. and Comm. VIII e Congres Int. Bot., Paris*, 1954, **17**, 122–24.

—— and Markali, J. .. "Morpholcgy and microstructure of Coccoliths studied in the electron microscope. Observations on *Anthosphæra robusta* and *Calyptrosphæra pcpillifera*," *Nytt. Mag. Bot.*, 1954 *a*, **2**, 117–18.

———— .. "Observations on the coccoliths of *Syracosphæra mediterranea* Lohm., *S. pulchra* Lohm., and *S. molischi* in the electron microscope," *J. Cons. int. Explor. Mer.*, 1954 *b*, **19** (3), 329–36.

Halledal, P. and Markali, J. .. "Electron microscope studies on Coccolithophorids from the Norwegian Sea, the Gulf Stream and the Mediterranean," *Avh. norske Videnskakad.*, 1955, *n.* 1, pp. 30.

Helmcke, J. G. .. "Die Feinstruktur der Kieselsaure und ihre physiologische Bedeutung in Diatomeenschalen," *Naturwisschenschaften*, 1954, **41**, 254–55.

—— and Krieger, W. .. "Feinbau von Diatomeenschalen in Einzeldarstellungen. 1. *Cocconies placentula*," *Z. wiss. Mikr.*, 1951 *a*, **60**, 85–100.

———— .. "Feinbau von Diatomeenschalen in Einzeldarstellungen. 2. Die Gattung *Achnanthes* Bory," *Ibid.*, 1951 *b*, **60** (3/4), 197–202.

———— .. "Demonstration einiger raumrichtiger Rekonstruktionszeichnungen von Diatomeenschalen," *Verh. dtsch. Zool. Ges., Wilhelmschaven*, 1951 *c*, 438–43.

———— .. "Elektronenmikroskopische Untersuchungen über den feinbau der Diatomeenmembran," *Ber. dtsch. Bot. Ges.*, 1951 *d*, **64**, 29–30.

———— .. "Feinbau der Kieselschalen der Diatomee *Cyclotella comta* (Ehrenb.) Kütz.," *Ibid.*, 1952 *a*, **65**, 70–72.

———— .. "Neue Erkenntnisse über dem Schalenbau von Diatomeen," *Naturwisschenschaften*, 1952 *b*, **39**, 146–49.

———— .. "Feinbau von Diatomeenschalen in Einzeldarstellungen. 3. Die Gattung *Melosira*," *Ag. Z. wiss. Mikr.*, 1952 *c*, **61**, 83–92.

———— .. "Kieselalgen in Elektronenmikroskops," *Kosmos Stuttgart*, 1952 *d*, **48**, 405–10.

———— .. *Diatomeenschalen in Elektronenmikroskopischen Bild*, Bd. I, 1953, Bd. II, 1954 *a*, Atlas, 201 pl. with text. Transmare-Photo, Gm. B.H., Berlin.

———— .. "Elektronenmikroskopische Untersuchungen über Feinstrukturen der Diatomeenschalen," *VIII e Congres Int. Bot., Rapp. et Comm.*, 1954 *b*, Sect. **17**, 126–27.

Hendey, N. I. .. "Note on the Plymouth '*Nitzschia*' culture," *J. Mar. biol. Ass., U.K.*, 1954, **33** (2), 335–39.

Houwink, A. L. .. "Die Pellikularschuppen und die Geisel der *Physomonas vestita* Stokes," *Z. wiss. Mikr.*, 1951, **60**, 402–04.

———— .. "An EM study of the flagellum of *Euglena gracilis*," *Proc. K. Nad. Akad. Wetenschap.*, 1951, Ser. C, **54**, 132–37.

Hustedt, F. .. "Die Struktur der Diatomeen und die Bedeutung des Elektronenmikroskops für ihre Analyse," *Arch. Hydrobiol. (u. Plakton.)*, 1945, **41**, 315–31.

———— .. "Die Struktur der Diatomeen und die Bedeutung des Elektronenmikroskops für ihre Analyse, II," *Ibid.*, **47** (2), 1952, 295–301.

———— .. "Die Grundsatzliche Struktur der Diatomeen-Membran und die taxonomische Auswertung elektronemnikroskopischer Diatomeenaufnahmen," *Bot. Notiser.*, 1955, **108** (5), 446–60.

Kamptner, E. .. "Das mikroskopische Studien des Skelettes der Coccolithineen (Kalkflagellaten). II, Der Feinbau der Coccolithen," *Mikroskopie*, 1952, **7**, 375–86.

K lbe, R. W. .. "Elektronenmikroskopische Untersuchungen von Diatomeenmembran. I." *Ark. Bot.*, 1948, **33 A** (17), 1–21.

———— .. "Elektronenmikroskopische Untersuchungen von Diatomeenmembran, II," *Svensk. bot. Tidsskr.*, 1951, **45** (4), 635–47.

———— .. "Einige Bemerkungen zu drei Aufsatzen von Fr. Hustedt," *Bot. Notiser*, 1954, **43**, 2*i*, 7–229.

———— .. "Zur Deutung und Answertung electronenmikroskopischer Aufnahmen in der Diatomeenkunde," *Bot. Notiser*, 1956, **109** (3), 368–73.

——— and Gölz, E. .. "Elektronenoptische Diatomeenstudien," *Ber. dtsch. bot. Ges.*, 1943, **51**, 93–98.

Lewin, R. A. and Meinhart, J. O. "Studies on the flagella of Algæ. III. Electron micrographs of *Chlamydomonas Mœwusii*," *Canad. J. Bot.*, 1953, **31**, 711–17.

Leyon, H. .. "The structure of the chloroplast. III. A study of Pyrenoids," *Exp. Cell. Res.*, 1954, **6**, 497–505.

Lhotsky .. "The pore system of the Desmidiaceæ," *Experentia*, 1948, **4**, 158.

Manton, I. .. "The fine structure of plant cilia. Structural aspects of cell Physiology," *Soc. exp. Biol.*, 1952, Symposia, **6**, 306–19.

———— .. "Number of fibrils in the cilia of the green algæ," *Nature, Lond.*, 1953, **171**, 485–86.

———— .. "Observations with the electron microscope on *Synura caroliniana* Whitford," *Proc. Leeds phil. lit. Soc.*, 1955, **6**, 306–16.

———— and Clarke, B. .. "Electron microscope observations on the zoospores of *Pylaiella* and *Laminaria*," *J. exp. Bot.*, 1951 *a*, 242–46.

———— .. "An electron microscope study of the spermatozoid of *Fucus serratus*," *Ann. Bot. Lond.*, 1951 *b*, **n.s. 15**, 461–71.

——— and Greenwood, A. .. "Observations with the Electron microscope on biciliate and quadriciliate zoospores in green algæ," *J. exp. Bot.*, 1955, **6** (16), 126–28.

———— .. "Further observations with the electron microscope on spermatozoids in brown algæ," *Ibid.*, 1953, **4**, 319–29.

——— and Flint, E. A. .. "Further observations on the structure of plant cilia, by a combination of visual and electron microscopy," *Ibid.*, 1952, **3**, 204–15.

Markali, J. and Paasche, E. .. "On two species of *Umbellosphæra*, a new marine Coccolithophorid genus," *Nytt. Mag. Bot.*, 1955, **4**, 95–98.

Moner, J. C. .. "Cell-wall structure in *Pediastrum* as revealed by electron microscopy," *Amer. J. Bot.*, 1955, **42** (9), 802–6.

Muhlethaler, K. .. "Submikroskopische Morphologie," *Fortschr. Bot.*, 1954, **16**, 72–95.

Okuno, H. .. 'Electron microscopical study on fine structures of diatom frustules, I," *Kagaku, Tokyo*, 1944 *a*, **14**, 166–69.

Okuno, H. .. "Electron microscopical study on fine structures of diatom frustules, II." *Kagaku, Tokyo*, 1944 *b*, **14**, 305–10.

——— .. "Electron microscopical study on fine structures of diatom frustules, III," *Ibid.*, 1947, **17**, 307–12.

——— .. "Electron microscopic study on diatom frustules, I," *Schimadzu Review*, 1948 *a*, **5**, 45–48.

——— .. "Electron microscopic study on diatom frustules, I," *Ibid.*, 1948 *b*, **5**, 100–04.

——— .. "Electron microscopic study on fine structures of diatom frustules, VI," *Bot. Mag., Tokyo*, 1949 *a*, **62**, 97–100.

——— .. "Electron microscopic study on fine structures of diatom frustules, VII," *Ibid.*, 1949 *b*, **62**, 136–40.

——— .. "On the electron microscopical fine structure of *Pinnularia* cell-wall," *Ibid.*, 1950 *a*, **63**, 34–35.

——— .. "On the electron microscopical fine structure of fossil *Coscinodiscus occulus-iridis*. Ehr.," *Ibid.*, 1950 *b*, **63**, 232.

——— .. "Electron microscopical study of fine structures of diatom frustules, VIII," *Ibid.*, 1950 *c*, **63**, 97–106.

——— .. "Electron microscopical study of antarctic diatoms, I," *J. Jap. Bot.*, 1951 *a*, **26** (10), 305–10.

——— .. "Electron microscopical study of antarctic diatoms, II," *Ibid.*, 1952 *a*, **27** (2), 46–52.

——— .. "Electron microscopical study of antarctic diatoms, III," *Ibid.*, 1952 *b*, **27** (11), 347–56.

——— .. "Electron microscopical study on fine structures of diatom frustules, IX," *Bot. Mag., Tokyo*, 1952 *c*, **65**, 158–63.

——— .. *Atlas of Fossil Diatoms from Japanese Diatomite Deposits*, H. Okuno, Japan, 1952 *d*, pp. 49.

——— .. "Electron microscopical study of antarctic diatoms, IV," *J. Jap. Bot.*, 1953 *a*, **28** (6), 171–78.

——— .. "Electron microscopical study of fine structures of diatom frustules, X," *Bot. Mag., Tokyo*, 1953 *b*, **66**, 5–8.

——— .. "Electron microscopical study of fine structures of diatom frustules, XI," *Ibid.*, 1953 *c*, **66**, 121–24.

——— .. "Study on superfine structure of fossil diatoms," *Proc. 1st Int. Cong. Electron Microscopy*, 1953 *d*, 758.

——— .. "Electron microscopical study of antarctic diatoms, V," *J. Jap. Bot.*, 1954 *a*, **29** (1), 18–25.

——— .. "Electron microscopical study of fine structures of diatom frustules, XII," *Bot. Mag., Tokyo*, 1954 *b*, **67**, 172–77.

——— .. "Electron microscopic study of fine structures of some marine diatoms," *Rev. Cyt. Biol. Vege.*, 1954 *c*, **15** (3), 237–44.

——— .. "Electron microscopic study of fine structure of fossil diatoms, I," *Trans. Proc. Palæont. Soc. Japan, N.S.*, 1954 *d*, **13**, 125–30.

——— .. "Electron microscopic fine structure of fossil diatoms, II," *Ibid.*, 1954 *e*, **14**, 143–48.

Okuno, H. .. "Four marine diatoms under electron microscope," *VIII e Congres Int. Bot., Paris., Rapp. et Comm.*, 1954 *f*, Sec. **17**, 124–25.

———— .. "Electron microscopical study on fine structures of Diatom frustules, XIII," *Bot. Mag., Tokyo*, 1955 *a*, **68**, 125–28.

———— .. "Fine Structures of Diatom Frustules," *Sobi*, 1955 *b*, *n.* **21**, 1–7.

Parke, M., Manton, I. and Clarke, B. "Studies on marine flagellates. II. Three new species of *Chrysochromulina*," *J. Mar. biol. Ass. U.K.*, 1954, **34**, 579-609.

———— .. "Studies on marine flagellates. III. Three further species of *Chrysochromulina*," *Ibid.*, 1956, **35**, 387–414.

Petersen, J. B. and Hansen, J. B. "Electron microscope observations on *Codonosigia botrytis* (Ehre)," J. Clark, *Bot. Tidsskr.*, 1954, **51**, 281–91.

———— .. "On the scales of some *Synura* species," *K. Danske Vidensk. Selsk. Biol. Medd.*, 1956, **23** (2), 1–27.

Preston, R. D. .. *The Molecular Architecture and Plant Cell-Walls*, London, 1954, 211 pp.

——— and Kuyper, B. .. "Electron microscopic investigations of the walls of green algæ, I," *J. exp. Bot.*, 1951, **2** (5), 247–55.

———, Nicolai, E. and Kuyper, E. "Electron microscopic investigations of the walls of green algæ, II. The cytoplasm-wall relationship in *Valonia macrophysa*," *Ibid.*, 1953, **4** (10), 40–43.

———, Reed, R. and Millard, A. "An electron microscope study of cellulose in the wall of *Valonia ventricosa*," *Nature, Lond.*, 1948, **162**, 665.

Schiller, J. .. *Coccolithineæ in Rabenhorst's Kryptogamenflora*, **10** (2), 89–273, Leipzig.

Singh, R. N. .. "Electron micrographs of the mucilage of the Blue-green algæ, I. *Scytonema pseudoguayanense*," *Proc. European Congr. Electron Microscopy, Ghent, Belgium*, 1954.

Steinmann, E. .. "An electron microscope study of the lamellar structure of the chloroplasts," *Exp. Cell. Res.*, 1952, **3**, 367–72.

Toman, M. and Rozsival, M. .. "The structure of the raphe of *Nitzschia*," *Studia bot. cechosl.*, 1948, **9**, 26–29.

Wolken, J. J. and Palade, G. E. "Fine structure of chloroplasts in two flagellates," *Nature, Lond.*, 1952, **170**, 114–15.

———— .. "An electron microscope study of two flagellates. Chloroplast structure and variation," *Ann. N.Y. Acad. Sci.*, 1953, **56**, 873.

TRANSLOCATION OF GRISEOFULVIN IN RICE SEEDLINGS (*ORYZA SATIVA* L.)

By N. S. Subba-Rao

(*University Botany Laboratory, Madras-5*)

Received May 3, 1957

(Communicated by Prof. T. S. Sadasivan, F.A.SC.)

Introduction

A metabolic product of *Penicillium janczewskii* Zal. was isolated from *in vitro* cultures of the fungus and named ' Curling factor ', since it caused marked abnormalities in the development of hyphæ of *Botrytis alli* and other fungi (Brian, Curtis and Hemming, 1946). This substance was later found to be chemically and biologically identical with griseofulvin, the metabolic product of *Penicillium griseofulvum* Dierckx (Oxford, Raistrick and Simonart, 1939; Grove and McGowan, 1947; Brian, Curtis and Hemming, 1949). It is now known that when griseofulvin is applied to the roots of plants growing in culture solution, the substance could be detected in guttation drops (Brian *et al.*, 1946, 1949 *loc. cit.*; Brian, 1952; Stokes, 1954). The method of detection of griseofulvin is based on the degree of characteristic morphological response (stunting and curling) of the hyphæ of *Botrytis alli* to the presence of the antibiotic. In the present paper, a chromatographic method of detection of griseofulvin in guttation drops of rice seedlings (*Oryza sativa* L.; Adt. 4) has been described.

Material and Methods

The method of raising seedlings for studying the uptake of griseofulvin was essentially that of Stokes (*loc. cit.*). It consisted of germinating rice seeds on the top of a piece of cotton gauze held in position by rubber bands on 200 ml. pyrex beakers containing 1/10 Richard's synthetic medium without sucrose (pH 6·0). When the seedlings were seven days' old, the mineral solution was replaced by a fresh aliquot to which griseofulvin was added at concentration of 5 μg./ml. The plants transferred to fresh Richard's solution to which no griseofulvin was added formed the controls. The beakers were covered with bell-jars and incubated at room temperature (29–32° C.). The guttation fluids were collected with the aid of glass-capillaries at the following intervals after the plants were treated with the antibiotic: 6, 12, 24 and 30 hours. 0·1 ml. of the guttation fluids from both the control and griseofulvin-treated plants and a freshly prepared alcoholic solution of pure griseofulvin*

* A sample of pure griseofulvin was kindly supplied by Dr. P. W. Brian of I.C.I. Ltd., Akers Research Laboratories, the Frythe, Welwyn, Herts, England.

were chromatographed by the circular paper chromatographic technique (Lakshminarayanan, 1954) using three different solvent mixtures. The solvent mixtures were *n*-Butanol/Acetic acid/Water (4 : 1 : 5); *n*-Butanol/Formic acid/Water (10 : 2 : 5) and Phenol/Water (1 : 1). The developed chromatograms were air-dried, exposed to vapours of liquor ammonia for 3 minutes, heat-treated at 90–105° C. for 5 minutes, sprayed with a 0·2% solution of triketo-hydrindene hydrate in acetone, air-dried and finally heat-treated at 90–105° C. for 10 minutes.

RESULTS AND DISCUSSION

Sharp brown bands against a light pink background were visible indicating the position of griseofulvin on the filter-paper. The R_f values (Table I) of griseofulvin contained in the guttation fluid of the antibiotic-treated plants were identical with those of the pure substance in all the three solvent systems employed. The chromatograms of guttation fluid from the control plants did not reveal any band. By this technique, griseofulvin has been detected from the guttation drops of seven-day old griseofulvin-treated rice plants from the twelfth hour after the plants were allowed to take up the substance, indicating the rapidity with which the antibiotic moves along the transpiration stream.

TABLE I

R_f values of pure and translocated griseofulvin

Solvent mixture	R_f values	
	Pure griseofulvin	Translocated griseofulvin in guttation drops
n-Butanol/Acetic acid/Water : : 4 : 1 : 5	0·513	0·510
n-Butanol/Formic acid/Water : : 12 : 2 : 5	0·243	0·249
Phenol/Water : : 1 : 1	0·621	0·621

SUMMARY

A chromatographic method of detection of griseofulvin in guttation drops of rice seedlings (*Oryza sativa* L.) has been described. This technique has demonstrated that seven-day old seedlings translocate the antibiotic from the roots to the leaves in twelve hours.

ACKNOWLEDGEMENTS

I am grateful to Professor T. S. Sadasivan for guidance and Dr. C. V. Subramanian for his keen interest in the present investigation.

REFERENCES

Brian, P. W. .. "Antibiotics as systemic fungicides and bactericides," *Ann. appl. Biol.*, 1952, **39**, 434.

————, Curtis, P. J. and Hemming, H. G. "A substance causing abnormal development of fungal hyphæ produced by *Penicillium janczewskii* Zal. I. Biological assay, production and isolation of 'Curling factor'," *Trans. Brit. mycol. Soc.*, 1946, **29**, 173.

———— .. "A substance causing abnormal development of fungal hyphæ produced by *Penicillium janczewskii* Zal. III. Identity of 'Curling factor' with griseofulvin," *Ibid.*, 1949, **32**, 30.

Grove, J. F. and McGowan, J. C. "The identity of griseofulvin and 'Curling factor'," *Nature, Lond.*, 1947, **160**, 574.

Lakshminarayanan, K. .. "A simple technique in paper disk chromatography," *Arch. Biochem. and Biophys.*, 1954, **49**, 396.

Oxford, A. E., Raistrick, H. and Simonart, P. "Studies in the biochemistry of micro-organisms. LX. Griseofulvin, $C_{17}H_{17}O_6Cl$, a metabolic product of *Penicillium griseofulvum* Dierckx," *Biochem. J.*, 1939, **33**, 240.

Stokes, A. .. "Uptake and translocation of griseofulvin by wheat seedlings," *Plant & Soil*, 1954, **5**, 132.

EMBRYOLOGICAL STUDIES IN COMPOSITÆ

III. *Gerbera jamesonii* Bolus

By H. Maheswari Devi

(*Department of Botany, Andhra University, Waltair*)

Received October 24, 1956

(Communicated by Prof. J. Venkateswarlu, F.A.SC.)

Introduction

This paper deals with *Gerbera jamesonii* Bolus belonging to the small tribe Mutisieæ, the embryology of which is little known.

Materials and Methods

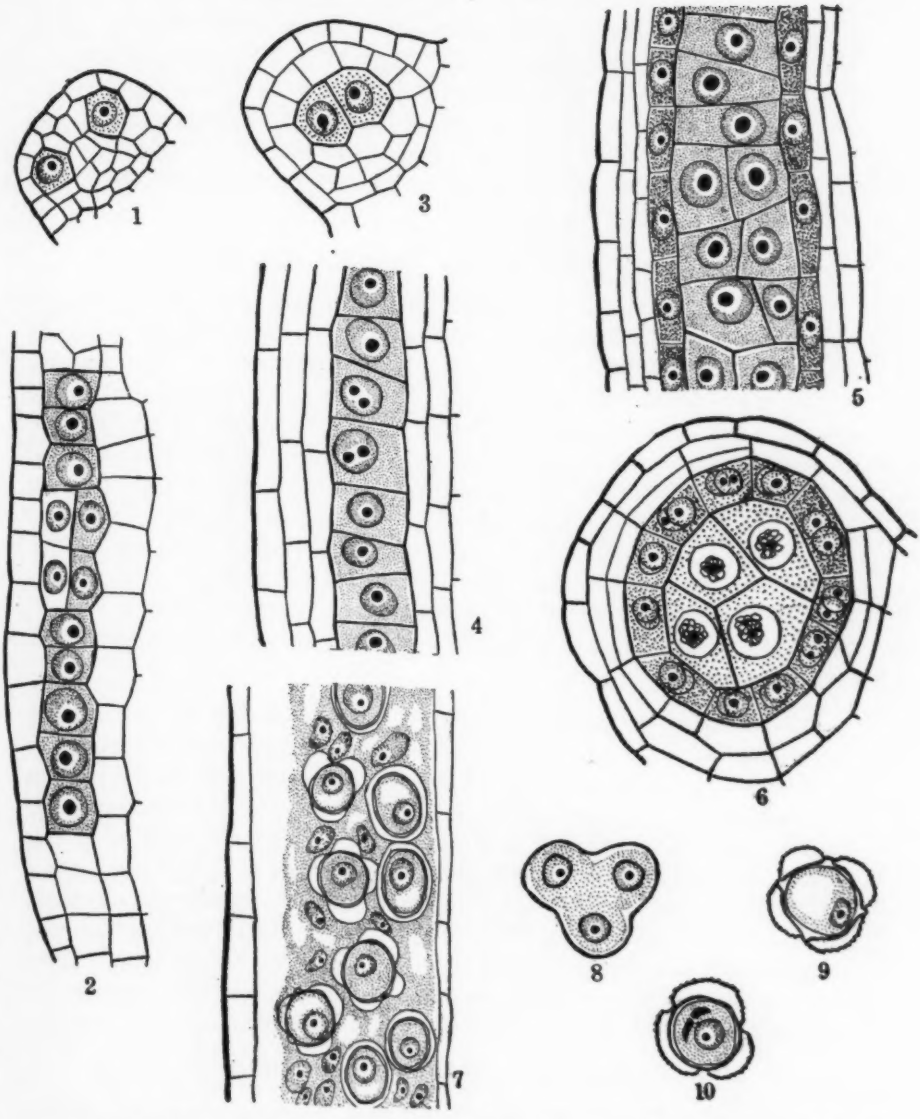
The material was obtained from plants cultivated in the Botanical Gardens of the Andhra University, Waltair. Either acetic-alcohol or formalin-acetic-alcohol was used as a fixative. Customary methods of dehydration and staining were followed. Sections were cut 8 to 12 microns thick and stained with Delafield's Hæmatoxylin.

Organogeny

The sequence of development of the floral whorls in bisexual flowers is petals, stamens and sepals followed by gynœcium. In female flowers also the same sequence is observed except that no stamens were developed.

Structure and Development of Anther and Pollen

The structure and development of the anther and pollen in *Gerbera jamesonii* presents features similar to those described in *Launæa pinnatifida* (Venkateswarlu and Maheswari Devi, 1955 *a*). The primary archesporium in each anther lobe consists of a row of eight to ten cells in longitudinal section and a single cell in transverse section (Figs. 1 and 2). In the structure of the anther, the epidermis is followed by two wall layers and a periplasmodial tapetum which surrounds the sporogenous tissue (Figs. 3 to 7). The sporogenous tissue is more extensive than in *Launæa pinnatifida* (Venkateswarlu and Maheswari Devi, 1955 *a*). In this respect and also in the structure and behaviour of the tapetal cells *Gerbera jamesonii* resembles *Tagetes patula* (Venkateswarlu and Maheswari Devi, 1955 *b*). Here also the parietal layers become crushed and disorganised during the development of the anther. The meiotic divisions in the pollen mother cell take place in a simultaneous manner and cytokinesis is by furrowing (Fig. 8). Pollen tetrads are either bilateral or tetrahedral.



TEXT-FIGS. 1–10. Structure and development of anther and pollen. Fig. 1. T.S. of an anther half showing primary archesporium. Fig. 2. L.S. of an anther lobe showing primary archesporium. Figs. 3–6. Anther lobes at various stages of development. Fig. 7. L.S. anther lobe showing periplasmodial tapetum surrounding the pollen grains. Fig. 8. Tetranucleate pollen mother cell showing cytokinesis by furrowing. Fig. 9. One-nucleate pollen grain showing four germ pores. Fig. 10. Mature three-celled pollen grain. Figs. 1–3 and 7, ×728; Figs. 4–6 and 8–10, ×1,091.

At the time of shedding the pollen grains are spherical and three-celled. The surface of the exine shows minute projections. There are three germ pores in it (Fig. 10). Occasionally pollen grains with four germ pores (Fig. 9) also have been encountered as in *Tagetes patula* (Venkateswarlu and Maheswari Devi, 1955 *b*), *Tridax procumbens* and *Blumea laciniata* (Banerjee, 1940 and 1942). No fibrous endothecium has been differentiated. The epidermis persists till maturity (Fig. 7).

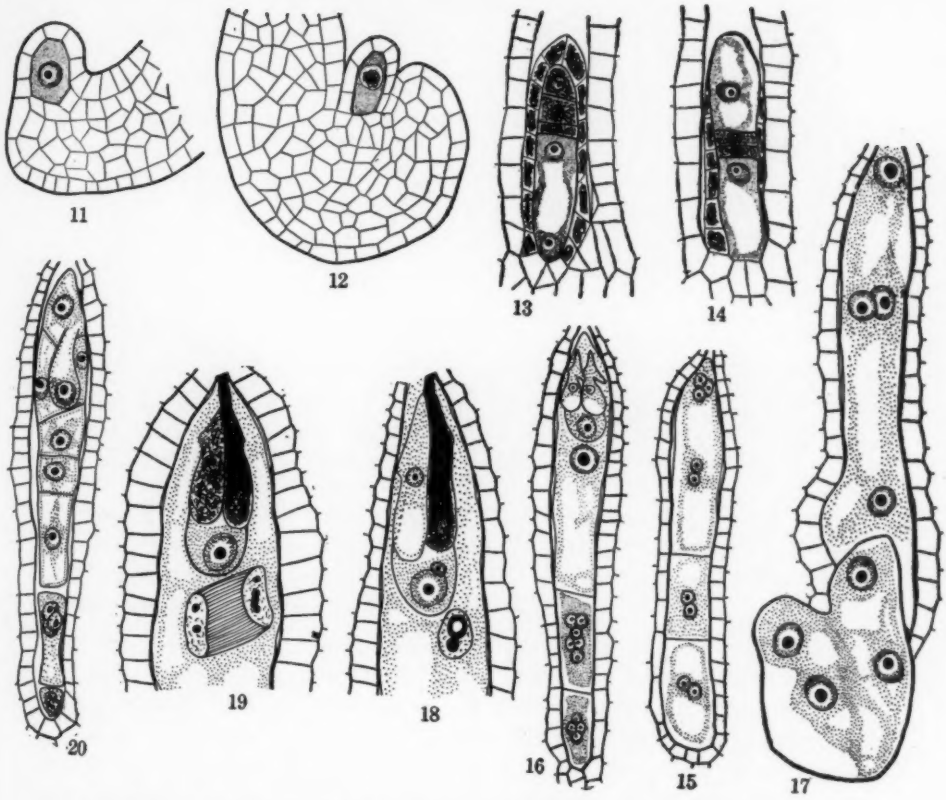
MEGASPOROGENESIS AND FEMALE GAMETOPHYTE

The ovule, as in all Compositæ, is anatropous, unitegmic and tenuinucellate. A single hypodermal archesporial cell is differentiated in the young ovule at about the time usually the integument is seen (Fig. 11). The archesporial cell enlarges and directly functions as the megaspore mother cell (Fig. 12). A linear tetrad of megaspores is formed (Fig. 13). Usually the chalazal megaspore becomes functional and an eight nucleate embryo-sac is formed according to the Polygonum type (Figs. 13, 15 and 16). In a few cases, however, both the micropylar and chalazal megaspores of the tetrad enlarge and develop upto the four-nucleate stage (Figs. 14 and 17) and the two middle ones degenerate. One of the cases is sketched in Fig. 17. In this the four nuclei are not distributed to each pole in the usual manner. In it one nucleus each is situated at either pole and two in the middle of the embryo-sac.

The cells of the nucellar epidermis become crushed by the growing embryo-sac and finally disappear by the time the eight-nucleate embryo-sac is formed. The innermost layer of the massive single integument becomes differentiated as the endothelium. In the eight-nucleate embryo-sac the organisation of the antipodal cells takes place earlier than those of the egg apparatus (Fig. 15). Usually only two antipodal cells are organised. About thirty embryo-sacs have been observed and all of them showed this condition. The number of nuclei in each of the antipodal cells varies from two to twelve. The antipodal cells persist upto the time the cotyledons become differentiated in the embryo. The synergids are hooked and the secondary embryo-sac nucleus is usually situated near the egg apparatus (Fig. 16).

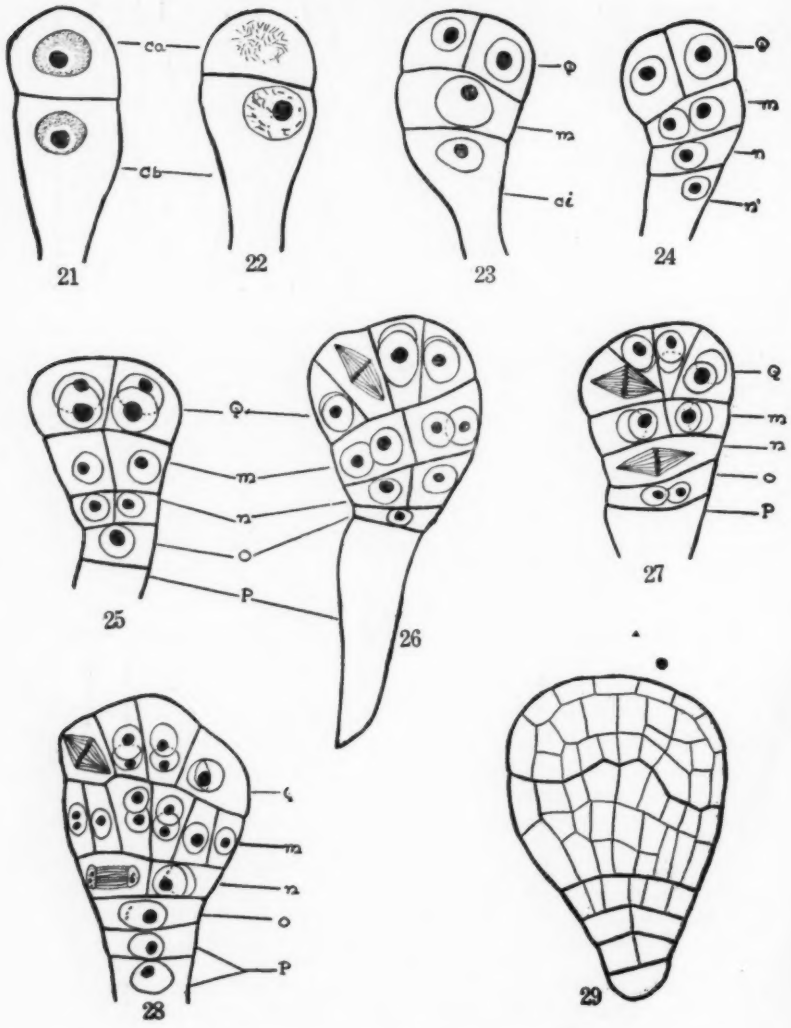
FERTILISATION, ENDOSPERM AND EMBRYO

Fertilisation is porogamous. The pollen tube discharges its contents into one of the synergids as in *Tagetes patula* (Venkateswarlu and Maheswari Devi, 1955 *b*). Syngamy and triple fusion take place normally (Fig. 18). The pollen tube and the synergids degenerate and disappear some time after the first division of the endosperm primordium.



TEXT-FIGS. 11–20. Structure and development of the female gametophyte. Figs. 11 and 12. L.S. young ovule showing archesporial cell and megaspore mother cell respectively. Fig. 13. Two-nucleate embryo-sac with the upper three degenerating megaspores. Fig. 14. Megaspore tetrad with chalazal and micropylar megaspores developing further. Figs. 15 and 16. Young and mature embryo-sacs. Fig. 17. Two 4-nucleate embryo-sacs. The nuclei are arranged irregularly. Fig. 18. Part of embryo-sac showing syngamy and triple fusion. Fig. 19. Upper part of embryo-sac showing the zygote, degenerating synergids and pollen tube. Endosperm nucleus is in its first division. Fig. 20. L.S. embryo-sac with egg, cellular endosperm and persisting antipodal cells. Figs. 11 and 12, ×339. Figs. 13, 14, 18 and 19, ×485. Figs. 15 and 16, ×242. Fig. 17, ×388. Fig. 20, ×172.

The endosperm is *ab initio* nuclear and the primary endosperm nucleus divides earlier than the fertilised egg (Fig. 19). The first division of the primary endosperm nucleus is sometimes transverse and other times vertical. In this feature *Gerbera jamesonii* resembles *Tagetes patula* (Venkateswarlu and Maheswari Devi, 1955 *b*). In later stages the endosperm becomes cellular (Fig. 20). It is completely absorbed in the mature seed.

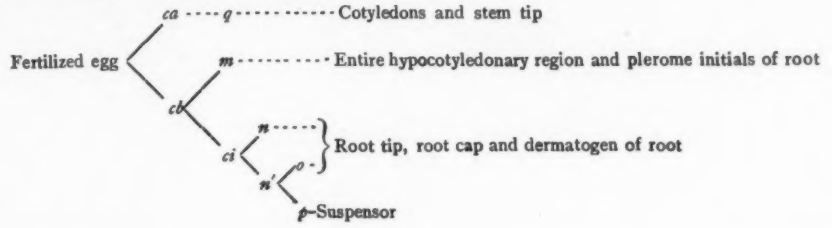


TEXT-FIGS. 21–29. Various stages in the development of the embryo, ×728.

ca and *cb*, apical and basal cells of the two-celled pro-embryo; *q*, Quadrants derived from *ca*; *m* and *ci*, upper and lower daughter cells arising out of the first division of *cb*; *n*, Upper daughter cell derived from *ci*; *n'*, Lower daughter cell derived from *ci*; *o* and *p*, Upper and lower daughter cells of *n'*.

The embryo development is closely similar to that in *Tagetes patula* (Venkateswarlu and Maheswari Devi, 1955 *b*) and is shown in Figs. 21 to 29

and in the following schematic representation. It conforms to the Asterad type and keys out to the Senecio variation.



Seed

In a fully developed seed the endosperm and also the integument, except for one or two of the outermost layers of its cells, are consumed by the embryo and thus the latter comes to lie in close proximity to the pericarp as in *Launæa pinnatifida* (Venkateswarlu and Maheswari Devi, 1955 *a*).

Summary

In bisexual flowers the organogeny follows the following sequence: petals, stamens, sepals and gynœcium and in the female flowers the sequence is the same but for the absence of the stamens.

The anther shows the epidermis, two wall layers, periplasmodial type of tapetum and sporogenous tissue. No fibrous endothecium is differentiated. The epidermis persists in the mature anther and the wall layers and periplasmodial tapetum disappear. Meiotic divisions in pollen mother cell are simultaneous. Cytokinesis is by furrowing. The mature pollen grains are three-celled. The exine shows small projections on the surface and contains three germ pores. Occasionally pollen grains with four germ pores have been encountered.

The ovule is unitegmic, tenuinucellate and anatropous. The primary archesporium is one-celled. It directly functions as the megaspore mother cell. Usually the chalazal megaspore of the linear tetrad develops into the eight-nucleate embryo-sac.

Fertilisation is porogamous.

Endosperm is of nuclear type. It later becomes cellular.

Embryo development conforms to the Asterad type and keys out to the Senecio variation.

The mature seed contains the fully developed embryo within the pericarp separated by a thin membrane which represents the crushed remains of the outer portion of the integument.

ACKNOWLEDGEMENTS

My grateful thanks are due to Prof. J. Venkateswarlu for kindly suggesting the problem and for critically going through the manuscript. I wish to express my sincere thanks also to Prof. Rev. Fr. H. Santapau for kindly furnishing me with the correct specific name of the plant.

REFERENCES

Banerji, I. .. "A contribution to the life-history of *Tridax procumbens* L.," *Proc. Ind. Acad. Sci.*, 1940, **1**, 325–36.

———— .. "A contribution to the life-history of *Blumea laciniata* L.," *Jour. Indian bot. Soc.*, 1942, **21**, 295–307.

Venkateswarlu, J. and Maheswari Devi, H. "Embryological studies in the Compositæ. I. *Launæa pinnatifida* Cass.," *Proc. Ind. Acad. Sci.*, 1955 *a*, **51**, 38–46.

———— .. "Embryological studies in Compositæ. II. Heleniæ," *Proc. Nat. Inst. Sci. India*, 1955 *b*, **21 B** (4), 149–61.

672–57. Printed at The Bangalore Press, Bangalore City, by C. Vasudeva Rao, Superintendent, and Published by The Indian Academy of Sciences, Bangalore.

CHILO TUMIDICOSTALIS HAMPSON—A SERIOUS STEM BORER PEST OF SUGARCANE IN BIHAR

By K. L. Khanna, F.A.Sc., L. N. Nigam and V. D. Puri
(Central Sugarcane Research Station, Pusa, Bihar)

Received March 25, 1957

1. Introduction

Pest survey is conducted in Bihar every year systematically, covering practically the whole of the sugarcane-growing area. During such a survey in 1940, a very severe and characteristic damage to mature canes by a stem borer was noticed in Purnea District. Numerous borer larvæ were found in each affected stalk, damaging a very large length of cane tissues. The species of borer responsible for this damage, though resembled closely *Chilo zonellus* Swinh. was, however, a different one. A detailed examination of larval and pupal characters established the identity of the pest as *Argyria tumidicostalis* or what is now known as *Chilo tumidicostalis* Hmps. This was the first record of the pest in Bihar.

The pest was earlier reported from Bengal and Assam, but unfortunately no work had been conducted on any aspect of this pest except that short descriptions of its adult, larva and pupa were given by Hampson (1919 *a*), Isaac and Rao (1941) and Isaac and Venkataraman (1941) respectively. Fletcher and Ghosh (1919) have also given a brief description of four different forms of its larvæ and pupa, placing the same under *Diatræa* species, which was named by Hampson as *A. tumidicostalis*.

The present paper gives in detail the distribution of the pest in Bihar along with its biology, nature and extent of damage and the natural enemies.

II. Historical

Cotes (1889) was the first who attempted to give specific name to the sugarcane moth borers in India. He erroneously named all the borers as *Diatræa saccharalis* Fb. But being aware of the unsatisfactory systematic position of the Indian species the same were sent to Dr. Riley, who identified these as species of *Chilo* nearing *C. plejadellus* Zinck or *C. infuscatellus* Snell. Hampson (1919 *a* and *b*) described the adult of *Argyria tumidicostalis* recorded from Bengal and *Chilo gemininotalis* recorded from Assam. Kapur (1950) re-examined the material from Assam and Bengal and opined that both these species were synonyms and should be renamed as *Chilo tumidicostalis* Hampson.

III. MATERIAL AND METHOD

A preliminary survey of this borer was conducted in various villages (21–30) of Purnea District for three years, 1939–42. A detailed survey was undertaken during the last three years (1953-56) to record the intensity of the borer in sugarcane-growing areas of Purnea and Darbhanga Districts with a view to studying the distribution of the pest and the trend of its migration. The method of recording pest incidence was adopted after Khanna and Bandyopadhyay (1951).

For studying the biology of this pest, a large number of its larvæ was collected from Purnea and Darbhanga Districts and reared in the laboratory at Pusa during September to February for the last three years (1954–56). The pest was reared on cane pieces which were changed regularly to avoid the infection by fungi.

Permanent slides of larvæ and adults were prepared by treating the specimens with 10% KOH solution, washing under a current of water, dehydrating, staining with eosin in 90% alcohol, clearing in carbo-turpentine, turpentine and cedarwood oil as suggested by Puri (1954) and finally mounting in turpentine-balsam.

IV. DISTRIBUTION

The pest is widely distributed all over Assam and Bengal States from where it was reported as early as 1907 and 1911 respectively. Recently, it has also been recorded in Bihar and Orissa. In Bihar, the pest is common only in Purnea District, though stray cases of its occurrence have also been recorded from North Bhagalpur, North Monghyr, Darbhanga and Muzaffarpur Districts. A detailed survey in Purnea and Darbhanga Districts revealed the spreading tendency of the pest (Plate IV). The concentration of the pest in North-East Bihar with gradual reduction in its intensity as it moves westwards clearly indicate that the pest has migrated from Bengal. This is a case where the pest has entered Bihar in cane supplies coming to the Semapur factory from affected areas and shows the danger of such importations to the industry.

V. INCIDENCE OF PEST

The pest survey during the earlier years showed that the incidence of this borer was very high in the factory plantation at Semapur and in the neighbouring villages. During 1940–41, the infestation in Semapur factory farms was, on an average, 82·18%, 14·34% and 11·98% on stalk, internodal and length basis respectively (Table II). The average infestation of this pest in different localities surveyed, was, in respect of stalk, joints and length bases, 29·76%, 3·53% and 2·57% during 1939–40 (Table I); 53·09%, 8·64%

TABLE I

Incidence of Chilo tumidicostalis *in Purnea District*, 1939–40 (*Co* 213)

Sl. No.	Name of villages	Percentage incidence on		
		Stalk basis	Length basis	Joint basis
1	Sakraili ..	41·40±3·09	4·40±0·16	5·80±0·37
2	Binji ..	30·68±2·82	3·50±0·14	4·12±0·32
3	Phulwaria ..	16·38±2·02	1·43±0·13	2·14±0·19
4	Barari ..	29·36±2·95	2·45±0·10	3·30±0·23
5	Sewana ..	23·05±2·54	2·18±0·11	2·83±0·21
6	Santari ..	21·85±2·43	2·57±0·13	3·12±0·26
7	Mednipur ..	36·46±2·97	4·70±0·21	5·66±0·35
8	Baghwara ..	37·41±3·00	4·25±0·18	4·09±0·27
9	Hanswar ..	46·65±3·32	4·62±0·25	6·30±0·41
10	Kishanpur ..	46·20±3·21	2·39±0·12	2·94±0·23
11	Lava ..	50·35±3·54	3·43±0·14	7·68±0·45
12	Lalgunj ..	59·13±3·41	4·54±0·21	2·70±0·18
13	Damka ..	55·20±3·36	6·10±0·32	8·93±0·49
14	Barsauni ..	31·55±2·92	2·72±0·11	3·81±0·25
15	Jalalgarh ..	57·53±3·15	8·24±0·26	11·55±0·55
16	Chilhania ..	31·69±3·01	2·65±0·18	4·46±0·31
17	Ararya ..	25·65±2·75	2·42±0·16	2·98±0·22
18	Dhama ..	2·15±0·82	0·21±0·04	0·44±0·06
19	Banmakhi ..	26·33±2·63	2·32±0·15	3·62±0·24
20	Kajhi ..	13·53±1·97	0·96±0·03	1·15±0·15
21	Mahadeopur ..	8·88±1·63	0·08±0·01	0·67±0·08
22	Barharakothi ..	25·44±2·56	1·16±0·08	2·39±0·18
23	Ramnagar ..	20·28±2·33	1·31±0·09	2·19±0·19
24	Rampur ..	21·82±2·40	1·05±0·06	1·35±0·14
	Overall ..	29·76±0·57	2·57±0·03	3·53±0·05

and 7·37% during 1940–41 (Table II) and 11·95%, 1·35% and 1·31% during 1941–42 (Table III). The incidence of this pest in different localities in Purnea and Darbhanga Districts surveyed variety-wise during 1953–54, 1954–55 and 1955–56 has been presented in Tables IV, V and VI respectively.

VI. VARIETAL RESISTANCE

The behaviour of the borer towards the different varieties was studied during three alternate seasons 1940–41, 1942–43 and 1944–45 and three successive seasons 1953–54 to 1955–56. The mean incidences on stalk basis in respect of different varieties obtained in the samples during the different seasons have been furnished in Tables VII and VIII (*a*, *b* and *c*) which clearly bring out the varietal differences. B.O. 10 is least affected on the three basis of infestation, *viz.*, stalk, joints and length bored while Co 421 is the most

TABLE II

Incidence of Chilo tumidicostalis *in Purnea District*, 1940–41 (*Co* 213)

Sl. No.	Name of villages	Percentage incidence on		
		Stalk basis	Length basis	Joint basis
1	Horprasad ..	60·38±3·09	12·18±0·25	13·43±0·55
2	Mednipur ..	56·38±3·18	6·72±0·18	7·96±0·42
3	Hasina ..	55·55±3·10	8·33±0·21	9·03±0·46
4	Balthi ..	48·28±3·19	5·79±0·18	10·33±0·49
5	Saturi ..	46·98±3·25	4·99±0·15	6·64±0·42
6	Chitabhari ..	43·65±3·11	6·97±0·26	6·31±0·41
7	Kaira ..	51·95±3·01	7·44±0·13	7·22±0·48
8	Mangera ..	43·54±3·88	5·79±0·35	6·82±0·51
9	Semapur (Zirat)	82·18±2·43	11·98±0·22	14·34±0·53
10	Nayatola ..	48·77±3·19	5·83±0·21	6·42±0·39
11	Katilpur ..	56·77±2·98	6·47±0·24	7·52±0·44
12	Lava ..	52·75±2·89	6·22±0·20	7·24±0·38
13	Kumaripur ..	56·40±2·92	8·88±0·28	10·52±0·49
14	Nawabganj ..	35·97±3·05	3·08±0·11	4·40±0·31
15	Noranji ..	64·51±3·11	10·05±0·15	12·42±0·46
16	Sakraili ..	51·03±2·93	7·28±0·17	8·14±0·44
17	Purnea Farm ..	63·60±3·04	8·31±0·26	10·29±0·49
18	Kosha ..	44·98±2·92	5·29±0·23	7·00±0·41
19	Ajampur ..	52·98±3·13	7·85±0·25	8·88±0·45
20	Budhuchak ..	57·08±3·07	8·15±0·15	9·18±0·46
21	Kantakosh ..	39·08±3·12	4·23±0·12	5·65±0·38
	Overall ..	53·09±0·69	7·37±0·04	8·64±0·10

affected, varieties Co 513, Co 356 and B.O. 11 coming near B.O. 10 and varieties Co 313, Co 331, Co 453 and Co 299 that of Co 421. The former group was generally solid-cored while the latter developed considerable pith and cavity in their stalks.

VII. ENVIRONMENTAL EFFECTS

Three effects of different environmental factors, *viz.*, type of soil, its susceptibility to water-logging and flooding and nature of the crop whether ratoon and plant on the activity of the pest were studied in the different villages. The result (Table IX) showed that the incidence of this pest was significantly higher in heavy soil as also under water-logged and flooded conditions suggesting that conditions which afforded more humid environment were more favourable for its multiplication. Further the ratoon crop showed significantly higher incidence than the plant crop.

TABLE III

Incidence of Chilo tumidicostalis *in Purnea District*, 1941–42 (*Co* 213)

Sl. No.	Name of villages	Percentage incidence on		
		Stalk basis	Length basis	Joint basis
1	Basalgaon ..	8·30±2·13	0·89±0·09	1·07±0 20
2	Niridhar ..	11·11±1·37	0·50±0·411	0·78±0·26
3	Geradih ..	10·90±1·00	0·87±0·060	1·33±0·28
4	Bilaspur ..	6·90±2·36	0·32±0·020	0·46±0·15
5	Kaushal ..	16·66±2·88	1·66±0·190	1·63±0·35
6	Bawanganj ..	14·30±2·96	0·99±0·100	1·33±0·22
7	Birja ..	0·00	0·00	0·00
8	Sakraili ..	3·60±1·77	0·22±0·010	0·23±0·11
9	Dharhara ..	2·73±4·60	0·21±0·010	0·29±0·09
10	Dhina ..	0·00	0·00	0·00
11	Parhari ..	32·00±3·82	0·17±0·010	0·85±0·30
12	Santari ..	10·00±2·54	0·17±0·007	0·35±0·09
13	Bantori ..	5·50±2·65	0·33±0·070	0·54±0·27
14	Husaina ..	5·30±1·29	0·39±0·080	0·53±0·14
15	Habibganj ..	6·58±2·02	0·35±0·010	0·45±0·17
16	Kursella ..	0·00	0·00	0·00
17	Baghmera ..	23·21±4·01	1·59±0·010	2·65±0·36
18	Mednipur ..	10·17±2·79	0·67±0·008	0·53±0·16
19	Bagahar ..	7·35±3·16	0·64±0·008	1·01±0·20
20	Matihari ..	18·33±3·55	1·98±0·010	2·81±0·39
21	Halbaidi ..	14·52±3·66	1·82±0·010	2·02±0·31
22	Mahadeopur ..	27·63±3·64	3·32±0·010	4·04±0·31
23	Bahadurpur ..	13·51±3·25	1·77±0·010	1·82±0·10
24	Keshopur ..	10·39±2·47	1·09±0·006	1·52±0·09
25	Anta Bigha ..	4·48±2·53	0·21±0·006	0·26±0·06
26	Purnea Farm ..	14·03±1·48	1·16±0·010	1·34±0·02
27	Girsole ..	25·00±5·38	4·36±0·010	4·29±0·45
28	Magura ..	10·81±5·11	1·11±0·010	1·60±0·39
29	Meraji ..	21·31±5·24	2·82±0·010	3·06±0·30
30	Maheshpur ..	21·11±4·30	2·38±0·008	2·30±0·34
	Overall ..	11·95±0·73	1·31±0·03	1·35±0·06

VIII. NATURE AND SYMPTOMS OF DAMAGE

The pest appears early in March when the damage by the first brood is not pronounced. The ravages of the pest really start with the second brood by August-September, when the crop is sufficiently old and have formed internodes. The attack continues till harvest. The larvæ usually prefer the upper half of the stems. A large number of larvæ are often found congregated in a few internodes. In quite a few cases as many as 40–60 larvæ were

TABLE IV

Incidence of Chilo tumidicostalis *in different varieties in Purnea District*, 1953–54

Sl. No.	Name of villages	Percentage infestation on		
		Stalk basis	Joint basis	Length basis
		Variety B.O. 11		
1	Purnea ..	22·22±5·66	3·69±0·59	2·22±0·22
2	Manihari ..	22·50±6·61	2·84±0·59	1·50±0·23
3	Forbesgunj ..	7·69±4·27	0·51±0·25	0·21±0·08
4	Semapur ..	10·13±2·00	8·55±0·40	0·36±0·04
5	Kishangunj ..	12·82±5·35	0·82±0·31	0·36±0·11
6	Jogbani ..	5·00±3·44	0·36±0·21	0·10±0·06
7	Araria ..	4·65±3·14	0·53±0·24	0·86±0·15
	Mean ..	11·60±3·47	4·85±0·39	0·67±0·09
		Variety Co 453		
1	Purnea ..	33·15±3·53	6·96±0·45	4·37±0·17
2	Manihari ..	26·21±3·06	7·07±0·40	4·99±0·17
3	Forbesgunj ..	23·68±6·89	2·05±0·51	1·03±0·19
4	Semapur ..	21·69±2·99	4·04±0·32	3·12±0·14
5	Kishangunj ..	20·69±4·38	2·44±0·39	1·72±0·16
6	Jogbani ..	10·26±1·98	0·90±0·14	0·35±0·04
7	Araria ..	23·91±6·29	4·78±0·75	2·11±0·24
8	Karahgola ..	14·29±5·40	5·11±0·81	3·98±0·35
	Mean ..	21·76±3·38	4·31±0·37	2·87±0·15
		Variety Co 513		
1	Purnea ..	29·41±4·94	4·04±0·52	2·13±0·19
2	Semapur ..	12·40±2·99	2·87±0·37	2·08±0·15
3	Kishangunj ..	14·04±3·25	1·57±0·29	0·71±0·09
4	Jogbani ..	4·43±1·63	0·37±0·11	0·14±0·03
5	Araria ..	13·51±5·62	1·30±0·49	0·42±0·13
6	Karahgola ..	7·14±3·97	0·88±0·36	0·49±0·13
7	Kusiargaon ..	19·05±4·28	3·80±0·50	2·50±0·19
	Mean ..	13·57±3·34	2·08±0·33	1·22±0·12
		Variety Co 313		
1	Manihari ..	29·72±5·31	3·70±0·54	1·38±0·16
2	Forbesgunj ..	35·63±3·47	9·29±0·48	7·59±0·21
3	Semapur ..	33·33±2·00	3·97±0·79	2·25±0·29
4	Jogbani ..	2·06±2·44	1·99±0·57	0·69±0·16
5	Araria ..	21·62±6·77	2·30±0·61	1·05±0·19
	Mean ..	29·75±3·93	6·29±0·54	4·55±0·20
		Variety B.O. 10		
1	Purnea ..	11·71±4·51	0·89±0·28	0·46±0·11
2	Jogbani ..	4·95±2·15	0·30±0·38	0·13±0·04
	Mean ..	7·22±2·94	0·50±0·35	0·24±0·06
		Variety Co 331		
1	Manihari ..	30·18±3·08	9·31±0·54	8·91±0·23
2	Semapur ..	2·60±1·86	3·19±0·48	1·48±0·17
	Mean ..	23·36±2·78	7·80±0·53	7·07±0·22

TABLE V

Incidence of Chilo tumidicostalis *in different varieties in Purnea District* 1954–55

Sl. No.	Name of villages	Percentage infestation on		
		Stalk basis	Joint basis	Length basis
		Variety Co 356		
1	Manihari ..	10·80±2·13	1·21±0·15	0·97±0·06
		Variety Co 331		
1	Manihari ..	17·65±4·14	2·69±0·40	1·94±0·17
		Variety B.O. 11		
1	Semapur ..	2·62±1·33	0·19±0·06	0·15±0·03

TABLE VI

Incidence of Chilo tumidicostalis *in Purnea and Darbhanga Districts in* 1955–56

Sl. No.	Name of villages	Percentage infestation on		
		Stalk basis	Joint basis	Length basis
		Variety Co 356		
1	Manihari ..	18·18±1·89	2·13±0·14	2·16±0·08
		Variety Co 513		
1	Sakri ..	10·27±1·78	1·66±0·19	0·93±0·07
		Variety Co 453		
1	Sakri ..	40·00±5·31	7·14±0·64	5·87±0·31
		Variety B.O. 11		
1	Sakri ..	34·89±1·88	5·36±0·18	4·59±0·09

recovered from 2–3 internodes of a single cane, while in majority of canes 5–10 larvæ and pupæ were met with in one cane. The maximum number of pupæ in a single internode was observed to be 14 at Purnea in 1953–54. The larvæ destroy the internodal tissues of the stalk and often reduce it to an almost hollow structure (Plates V and V A).

The damage of this borer may be recognised by the following symptoms: (1) Copious frass of the borer larvæ, looking like saw-dust, is seen sticking to the stem near the borer holes. Very often masses of this excreta are found

TABLE VII

Showing incidence of Chilo tumidicostalis *on different varieties*

Sl. No.	1 Varieties	2 Stalk basis	3 Joint basis	4 Length basis
1	Co 213	31·60±0·67	4·51±0·07	3·75±0·03
2	Co 313	29·75±3·93	6·29±0·54	4·55±0·20
3	Co 331	20·51±3·52	5·50±0·47	4·51±0·19
4	Co 356	14·50±2·01	1·67±0·15	1·57±0·07
5	Co 453	30·83±4·45	5·73±0·52	4·37±0·24
6	Co 513	11·92±2·68	1·87±0·27	1·08±0·09
7	B.O. 10	7·22±2·94	0·50±0·35	0·24±0·06
8	B.O. 11	16·37±2·40	3·47±0·25	1·80±0·08
9	Co 299	36·07±6·15	4·68±0·62	4·06±0·29
10	Co 421	59·26±9·46	7·48±1·05	6·79±0·47

deposited in between the stalk and the sheathing leaves (Plate V A). (2) In cases of severe infestation, the larvæ bore through the stem in such a way that the whole of the peripheral tissue below the rind is eaten up in the form of a ring and hence the portion above the ring gets easily broken by wind (Plate V, Fig. 1). (3) Due to the congregation of larvæ in the plants, several entry and exit holes are made by them and as many as 6–10 holes are not infrequently seen in one internode.

IX. EXTENT OF DAMAGE

Loss in tonnage.—Due to gregarious habit of the boring larvæ the internal cane tissue is almost completely destroyed. The canes often become hollow tubes and lose in weight heavily. In severe cases the upper hollow portions give way to high winds and either break away or hang down becoming dry in due course. The intensity of loss in tonnage has varied in different years and in different localities from 8·2% to 12·6% (Table X). Similarly different varieties have shown (Table XI) distinct variations in the magnitude of loss suffered, Co 419 showing the heaviest loss and Co 213 and Co 331, the least (Table XI). This is rather interesting as the incidence was rather high in both these varieties and the differences seemed due to comparatively harder rind and core compared to Co 419. So far different districts are concerned, losses have been much more pronounced in North Bhagalpur and North Monghyr than in the Purnea District (Table XII).

Loss in sugar.—As this borer destroys the storage tissues of the cane, the sucrose percentage of the juice is considerably reduced. From the samples collected in the different localities of Purnea District it was found (Table XIII)

TABLE VIII

Showing relative resistance to C. tumidicostalis *of different varieties*

(a) Stalk basis

	Co 313	Co 331	Co 356	Co 453	Co 513	B.O. 10	B.O. 11	Co 299	Co 421	Remarks
Co 213	1·85±3·99 N.S.	11·09±3·58†	17·10±2·12†	0·77±4·50 N.S.	19·68±2·76†	24·38±3·01†	15·23±2·50†	4·47±6·19 N.S.	27·66± 9·49†	
Co 313	..	9·24±5·27 N.S.	15·25±4·41†	1·08±5·94 N.S.	17·83±4·76†	22·53±4·90†	13·38±3·07†	6·32±7·29 N.S.	29·51±10·24†	
Co 331	..	..	6·01±4·05 N.S.	10·32±5·68 N.S.	8·59±4·43 N.S.	13·29±4·59*	4·14±4·27 N.S.	15·56±7·09*	38·75±10·09†	
Co 356	..	..	..	16·33±4·89†	2·58±3·35 N.S.	7·28±3·56*	1·87±3·13 N.S.	21·57±6·48†	44·76± 9·67†	
Co 453	..	..	..	..	18·91±5·19	23·61±5·33†	14·46±5·05†	5·24±7·59 N.S.	28·43±10·45†	
Co 513	..	..	..	..	..	4·70±3·99 N.S.	4·45±3·59 N.S.	24·15±6·79†	47·84± 9·83†	
B.O. 10	..	..	..	..	..	..	9·15±3·79*	28·85±6·82†	52·04± 9·91†	
B.O. 11	..	..	..	..	..	..	..	19·70±6·60†	42·89± 9·76†	
Co 299	..	..	..	..	..	..	..	..	23·19±11·28*	

* Significant at 5 % level. † Significant at 1 % level.

TABLE VIII (*Contd.*)

Incidence (Varietal resistance) in different varieties

(*b*) *Joint basis*

	Co 313	Co 331	Co 356	Co 453	Co 513	B.O. 10	B.O. 11	Co 299	Co 421	Remarks
Co 213	1·78±0·54†	0·99±0·47*	2·84±0·17†	1·22±0·52*	2·64±0·28†	4·01±0·36†	1·04±0·26†	0·17±0·62 N.S.	2·97±1·01†	
Co 313	..	0·79±0·72 N.S.	4·62±0·56†	0·56±0·75 N.S.	4·42±0·60†	5·79±0·64†	2·82±0·59†	1·61±0·82 N.S.	1·19±1·18 N.S.	
Co 331	..	..	3·83±0·49†	0·23±0·70 N.S.	3·63±0·54†	5·00±0·59†	2·03±0·53†	0·82±0·78 N.S.	1·98±1·15* N.S.	
Co 356	..	..	..	4·06±0·54†	0·20±0·31 N.S	1·17±0·38†	1·80±0·29†	3·01±0·63†	5·81±1·05†	
Co 453	..	..	..	..	3·86±0·59†	5·23±0·63†	2·26±0·58†	1·05±0·81 N.S.	1·75±1·17 N.S.	
Co 513	..	..	..	..	..	5·79±0·44†	1·60±0·37†	2·81±0·68†	5·61±1·09†	
B.O. 10	..	..	..	..	..	..	2·97±0·43†	4·18±0·71†	6·98±1·11†	
B.O. 11	..	..	..	..	..	..	..	1·21±0·67 N.S.	4·01±1·08†	
Co 299	..	..	..	..	..	..	..	..	2·80±1·22*	

Incidence (Varietal resistance) in different varieties
(c) Length basis

	Co 313	Co 331	Co 356	Co 453	Co 513	B.O. 10	B.O. 11	Co 299	Co 421	Remarks
Co 213	0·80±0·20†	0·76±0·19†	2·18±0·08†	0·62±0·24*	2·67±0·09†	3·51±0·06†	1·95±0·09†	0·31±0·29 N.S.	3·04±0·47†	
Co 313	..	0·04±0·28 N.S.	2·98±0·21†	0·18±0·31 N.S.	3·47±0·22†	4·31±0·21†	2·75±0·31†	0·29±0·35 N.S.	2·24±0·51†	
Co 331	..	..	2·94±0·20†	0·14±0·30 N.S.	3·43±0·21†	4·31±0·20†	2·71±0·21†	0·45±0·34 N.S.	2·28±0·50†	
Co 356	..	..	..	2·80±0·25†	0·49±0·11†	1·33±0·09†	0·23±0·10*	2·49±0·30†	5·22±0·48†	
Co 453	..	..	..	..	3·29±0·26†	4·13±0·25†	2·50±0·25†	0·31±0·38 N.S.	2·42±0·53†	
Co 513	..	..	..	..	..	0·84±0·11†	0·72±0·12†	2·98±0·30†	5·71±0·48†	
B.O. 10	..	..	..	..	..	..	1·56±0·10†	3·82±0·29†	6·55±0·47†	
B.O. 11	..	..	..	..	..	..	..	2·86±0·30†	4·99±0·48†	
Co 299	..	..	..	..	..	..	..	..	2·73±0·55†	

* Significant at 5% level. † Significant at 1% level.

TABLE IX

Showing incidence of borer under different environments

	Environments	Stalk basis	Joint basis	Length basis
Co 213	Plant	30·43 ±1·02	4·01*±0·10	2·82*±0·04
	Ratoon	36·83 ±2·07	6·05 ±0·25	4·85 ±0·12
	Water-logging	38·01*±1·35	5·49*±0·16	3·80 ±0·06
	Non-water-logging	25·89 ±1·18	3·51 ±0·12	2·66 ±0·05
	Flooding	37·59*±1·55	5·45*±0·18	3·59 ±0·07
	Non-flooding	28·38 ±1·10	3·86 ±0·11	2·97 ±0·05
	Heavy soil	48·39 ±8·97	9·85*±1·36	9·80 ±0·76
	Light soil	31·89 ±0·91	4·45 ±0·10	3·16 ±0·04

* Significant at 5% level.

TABLE X

Showing actual loss % in tonnage due to Chilo tumidicostalis

Variety: Co 213

(*a*) 1939–40

Name of sites	Average weight of healthy cane	Average weight of cane affected by stem borer	% loss in weight due to stem borer
Sakraili ..	13·9	13·5	2·9
Binji ..	13·5	12·8	5·2
Phulwaria ..	11·5	11·7	−1·7
Berari ..	14·6	14·0	4·1
Sewana ..	13·9	14·3	−2·9
Sautari ..	14·4	15·4	−6·9
Mednipur ..	16·8	17·1	−1·8
Baghmara ..	14·1	13·9	1·4
Kishanpur ..	13·8	13·1	5·1
Lalganj ..	14·8	15·2	−2·7
Damka ..	14·2	13·9	2·1
Barsauni ..	9·2	7·3	20·7
Jalalgarh ..	10·4	9·3	10·6
Chilhania ..	12·9	11·7	9·3
Araria ..	10·9	11·5	−5·5
Dhima ..	14·6	12·7	13·0
Banmankhi ..	14·5	11·0	24·1
Kajhi ..	11·3	9·8	13·2
Mahadeopur ..	12·2	7·3	40·2
Barharakothi ..	11·1	11·1	0·0
Ramnagar ..	11·3	9·0	20·4
Rampur ..	15·60	13·1	16·0
Average ..	13·3	12·1	9·0

TABLE X (*Contd.*)

(*b*) 1940–41

Name of sites	Average weight of healthy cane	Average weight of cane affected by stem borer	% loss in weight due to stem borer
Korha ..	8·3	7·6	8·4
Kajra ..	10·6	9·9	6·6
Azampur ..	9·1	7·8	14·3
Mangura ..	9·0	7·6	15·6
Budhuchak ..	11·2	10·8	3·6
Kabilpur ..	11·9	11·8	0·8
Nayatola ..	10·4	9·8	5·8
Amirabad ..	11·6	9·8	15·5
Kantakush ..	9·1	9·7	−6·6
Medhipur ..	13·8	13·7	0·7
Marangi ..	11·5	10·7	7·0
Kumaripur ..	9·3	8·8	5·4
Harprasad ..	10·1	7·8	20·8
Balthi ..	11·1	11·4	−1·8
Nawabganj ..	14·9	11·6	22·1
Average ..	10·8	9·9	8·2

(*c*) 1942–45

Name of sites	Average weight of healthy cane	Average weight of cane affected by stem borer	% loss in weight due to stem borer
Gamitola ..	8·9	8·1	9·0
Chitabari ..	10·6	8·6	18·9
Mangura ..	8·0	7·2	10·0
Marangi ..	12·6	13·4	−6·3
Baghmara ..	14·2	12·8	9·9
Mednipur ..	14·2	11·3	20·4
Baghar ..	12·9	10·6	17·8
Keshopur ..	8·0	6·1	23·8
Ghoradih ..	7·9	6·8	13·9
Bawanganj ..	12·3	11·0	11·3
Binji ..	6·5	3·4	47·77
Dhima ..	12·6	8·0	36·5
Panihari ..	11·1	11·9	−7·2
Sautari ..	14·2	13·7	3·5
Husina ..	11·2	10·2	8·9
Nawabganj ..	15·0	15·5	−3·3
Kursella ..	8·3	5·7	31·3
Average ..	11·0	9·7	12·6

TABLE XI

Showing loss in tonnage in different varieties due to Chilo tumidicostalis

Variety	Average weight of healthy cane	Average weight of cane affected by stem borer	% loss in weight due to stem borer
Co 213	11·37	10·68	6·07
Co 421	27·71	24·33	12·20
Co 331	11·44	10·67	6·73
B.O. 4	16·55	14·22	14·08
POJ 2878	18·05	14·58	19·22
Co 313	10·22	8·99	12·04
Co 210	5·88	5·09	13·44
Co 513	17·08	15·33	10·25
Co 299	9·59	8·79	8·34
Co 419	26·63	17·33	34·92

TABLE XII

Showing actual loss % in tonnage due to Chilo tumidicostalis *in different districts*

Variety: Co 213

1943–45

Name of sites	District	Average weight of healthy cane	Average weight of cane affected by stem borer	% loss in weight due to stem borer
Sibi	Darbhanga	7·61	6·60	13·27
Maharajganj	,,	12·72	12·08	5·03
Average ..		10·17	9·34	8·16
Morangi	Purnea	11·31	7·81	30·95
Sewana	,,	11·69	10·28	12·06
Bijai	,,	7·88	7·14	9·39
Nathpur	,,	10·00	9·50	5·00
Average ..		10·22	9·43	7·73
Latra	North Bhagalpur	13·12	8·42	35·82
Tulsipur	,,	8·06	7·00	13·15
Parbetta	,,	9·98	5·11	48·80
Ganeshpur	,,	8·64	5·20	39·81
Average ..		9·95	6·43	35·38
Teghra	North Monghyr	8·29	6·80	17·97
Pipra	,,	10·13	8·17	19·35
Average ..		9·21	7·49	18·68

TABLE XIII

Loss in sugar due to Chilo tumidicostalis *in different varieties*, 1940–41

Varieties	Localities	Healthy canes		Unhealthy canes		Difference of sucrose % (H—A)	% loss in sucrose on healthy juice
		Brix %	Sucrose % H	Brix %	Sucrose % A		
Co 213	Sakraili	17·1	14·37	12·7	9·28	5·09	35·42
	Kajra	17·0	14·26	13·0	9·64	4·62	32·47
	Ajampur	17·2	14·48	13·6	10·36	4·12	28·45
	Chitabari	18·3	15·68	13·7	10·48	5·20	33·16
	Mangura	17·7	15·03	12·2	8·68	6·35	42·25
	Budhuchak	17·7	15·03	15·9	13·03	2·00	13·57
	Kabilpur	17·2	14·48	13·0	9·64	4·84	33·43
	Nayatola	17·1	14·37	12·6	9·16	5·21	36·25
	Lava	18·7	16·11	14·9	11·88	4·23	26·28
	Amirabad	18·1	15·47	15·9	13·03	2·44	15·72
	Kantakush	17·3	14·59	15·3	12·34	2·25	15·42
	Madnipur	17·8	15·14	15·5	12·57	2·57	16·97
	Marangi	17·7	15·03	15·2	12·23	2·80	18·63
	Kumaripur	17·0	14·26	15·0	12·00	2·26	15·83
	Harprasad	17·2	14·48	15·0	12·00	2·48	17·13
	Purnea Farm	18·1	15·47	16·4	13·59	1·88	12·15
	Sautari	16·5	13·70	13·7	10·48	3·22	23·50
	Husina	17·3	14·59	14·9	11·88	2·71	18·57
	Balthi	17·7	15·03	14·0	10·83	4·20	27·94
	Nawabganj	18·5	13·90	13·5	10·24	5·66	35·59
	Korha	16·1	13·25	11·8	8·10	5·15	38·87
	Average ..	17·5	14·81	14·2	11·07	3·74	25·25
Co 313	Nawabganj	17·8	15·14	13·2	9·88	5·26	34·74
	Semapur zirat	17·1	14·37	12·6	9·16	5·21	36·26
	Nayatola	18·5	15·90	14·3	11·18	4·72	29·68
	Lava	19·1	16·54	16·5	13·70	2·84	17·17
	Kantakush	17·1	14·37	15·2	12·23	2·14	14·89
	Mednipur	19·2	16·65	16·7	13·92	2·73	16·39
	Marangi	18·5	15·90	16·5	13·70	2·20	13·84
	Kumaripur	17·4	14·70	15·6	12·68	2·02	13·74
	Purnea Farm	19·5	16·96	17·8	15·14	1·82	10·73
	Harprasad	16·8	14·04	13·9	10·71	3·33	23·72
	Average ..	17·9	15·25	15·20	12·23	3·02	19·80
Co 513	Semapur zirat	17·9	15·25	13·4	10·12	5·13	33·64
B.O. 4	Semapur zirat	17·9	15·25	12·3	8·80	6·45	42·30
POJ 2878	Semapur zirat	17·3	14·59	12·6	9·16	5·43	37·22
Co 299	Semapur zirat	17·2	14·48	11·2	7·45	7·03	48·55
	Mangura	17·4	14·70	12·7	9·28	5·42	36·87
Co 421	Purnea Farm	19·6	17·07	15·9	13·03	4·04	23·73
Co 331	Purnea farm	19·3	16·75	14·6	11·53	5·22	31·16

that the sucrose percentage in the juice of healthy canes of Co 213 was reduced on an average by 3·74 units due to the attack of this pest. The variation in the reduction of sucrose percentage ranged from 1·82 to 7·03 units. The average reduction in all the localities (3·74) when worked out on the basis of the sucrose percentage of pest-free juice came to be 25·25% in the area as a whole. In Co 313 the reduction varied from 1·82 to 5·26 with an average of 3·02 and percentage loss of 19·80. This pest thus constitutes a serious menace to the industry as it appears and damages the crop at a time when it is entering its maturation phase.

X. BIOLOGY

Adult (Plate VI, Figs. 6 and 7).—In September-October, freshly emerged moths from the field-collected pupæ copulated one day after their emergence and the females laid 3–4 egg clusters on the same day. The emergence of moths generally took place either between 5 P.M. and midnight or from 4 A.M. to 8 A.M. Under laboratory conditions, the copulation took on an average 45 minutes. Wing expanse of male and female were 21–26 mm. and 28–31 mm. respectively. The percentage of successful rearing from field collected larvæ upto adult stage was very low (6% only).

Egg (Plate VI, Figs. 1 *a* and 1 *b*).—Egg-masses consist of 3–4 rows of overlapping eggs, like the tiles of a roof. Eggs are flattened and whitish in colour when fresh. Next day, a blackish streak appears which deepens in colour and becomes prominent on the 4th day. It becomes very conspicuous on the 6th day and disappears on the 7th day, when a broad black spot develops which demarcates the head of the developing embryo. Each egg measures on an average 0·5 mm. in diameter. Most of the eggs, during the first fortnight of October, hatched after 8 days. The hatching usually took place in the morning hours and continued for 2–3 days in the same cluster. The incubation period varied from 8–11 days.

Larva (Plate VI, Figs. 2, 3 and 4).—Unlike the larvæ of other stem borers, the freshly hatched larvæ of this species are gregarious in habit and are very sluggish and inactive in movements. After hatching, they remain assembled in groups of 20–30 for a few hours, feeding upon the leaf surface and move away slowly towards the base of the leaf-lamina till they reach the inner portion of the leaf sheath and enter the central stalk from the side. They often come out, re-enter the other internodes and bore into the core of the stem making it almost hollow, in serious cases as shown in Plate V. The newly hatched larva is very hairy and presents a deep grey general appearance with a broad brown head, deeper in colour than the rest of the body. It measures on an average 1·5 mm.×0·5 mm. Four indistinct rows

of blackish spots or warts run longitudinally on the body almost parallel to one another. As the larva grows, these warts grow deeper in tinge and broader in size, making the rows very prominent. A full-grown larva measures on an average 25–27 mm. × 2–3 mm. The spiracles (Plate VI, Fig. 11) are open with a clear space in the centre and a black rim all round. The larvæ undergo 5–6 moults before pupation. During October and November the 1st, 2nd and 3rd moults were shed, each after a lapse of 3-5 days, whereas the 4th and the 5th moults were shed after a lapse of 2–3 weeks or even longer. The full ground larvæ hibernated during December to January, pupating in the first fortnight of February, when it resumed its activity. Some of the larvæ did not become active even upto March and died. The larval period varied from 61–121 days.

The larvæ exhibit a marked variation in their forms as reported earlier by Fletcher and Ghosh (*loc. cit*). These variations might be due to the climatic variations and have been observed to be exhibited by almost all the larvæ during the course of their development under laboratory and natural conditions. There are four different types of variations (Plate VI A). In one type, all the four longitudical stripes are distinct and pinkish-brown in colour resembling closely *Chilo zonellus* and *Chilotræa auricilia.* This form was collected in 1953–54 from Kishanganj area. The second type, which is the most common one, has the stripes very indistinct but the warts are prominent. These warts are brown in colour arranged in parallel rows on the indistinct stripes. Such a form has also been observed in the larvæ of *Chilo zonellus*, but the colour of the warts in the case of the latter is pinkish-brown. This form was collected from Purnea in 1953–54 and Sakri area in 1954–55. The third type has both warts as well as stripes indistinct. The proportion of this form is much less. The fourth type, as described by Fletcher and Ghosh (*loc. cit.*), possesses dark shining warts on the stripes, but the observations recorded by the present authors show that the stripes are arranged in the form of lateral brackets on each segment and are provided with minute warts on them. Such type of forms are the ones provided with deep brown-coloured warts are either parasitised or are those of the hibernating larvæ which never pupate but die away ultimately. The larvæ of *Chilo tumidicostalis* can be distinguished by the presence of open spiracles and triordinala crochets on the prolegs arranged in a complete circle (Plate VI, Fig. 10). The chætotoxy of the full-grown larvæ (Plate VI, Fig. 8) is more or less similar to that of *Chilo zonellus* as described by Trehan and Butani (1950).

Pupa (Plate VI, Figs. 5, 9 *a* and 9 *b*).—Freshly formed pupa is light-brown in colour, becoming darker after 4–5 days. The male and the female

pupæ are unequal in size, measuring on an average 10–12 mm. in the case of males and 15–17 mm. in case of females. It has a complete circle of distinct and separate double spines all round the seventh segment. On the 5th and 6th segments the spines are present in the form of an incomplete circle which opens ventrally. The 5–9 hooks at the caudal extremity are dark in colour. The genetal opening of the male and the female is situated on the 8th and 9th segments respectively. It is in the form of circular lobes in male and a slit-like apperture in female as shown in their respective figures. The pupal period lasts for 8–12 days during September to October, whereas during January to February it ranged between 18-26 days. The pupation takes place inside the tunnels in the stalks near the exit holes, cut by larvæ before pupation and through which the image comes out.

Number of broods.—In view of long period that larval stage takes it has not been possible to get more than two and occasionally three broods in a year, the second and the third brood not infrequently overlapping.

XI. NATURAL ENEMY AND ITS BIOLOGY

Large number of the larvæ of the borer were found parasitised by an Ichneumonid wasp, *Apanteles flavipes* which is endoparasitic in habit. The parasites are usually active during October and November and destroy a large population of the pest when much of the damage is already done. The adult parasites (Plate VII, Figs. 1 and 3) are very active and enter the stem through the borer holes to reach the host. Copulation takes place immediately after emergence from the cocoon (Plate VII, Fig. 5) and the female, soon after fertilization, starts laying eggs. The sexes can be easily separated from their size and form of antennæ which are filiform and comparatively longer in the males (Plate VII, Fig. 4) than in the females (Plate VII, Fig. 2).

The eggs are laid within the body cavity of the host larva which after parasitisation becomes sluggish, contracts in size and stops feeding. It continues to live for several days even after the emergence of the parasites. The longevity of such larvæ was found to range from 1–12 days. The eggs of the parasite hatch into grubs which feed within the body of the host till they become almost full grown. Then they come out and pupate in the larval tunnel near the host in white masses of elongated cocoons (Plate VII, Figs. 5 and 6) and (Plate VII A). As many as 35 or even more grubs have been found to emerge from a single host. The pupal period was 5–6 days during October and 10–15 days during November–December. The complete life-cycle took 25–30 days during November–December.

XII. Discussion

Chilo tumidicostalis—a serious pest of sugarcane had a limited distribution, being confined only to Bengal and Assam. When the Seemapur factory in Purnea District of Bihar was established in the early thirties and started getting part of its supplies from the adjoining areas of Bengal, this borer came along with them and in a matter of 3–4 years established itself in the sugar factory area. All sugarcane-growing villages in the district showed varying incidence of the pest as also damage to crop tonnage and quality of juice. Further its movement westward has been noticed in the northern parts of Bhagalpur, Monghyr and Darbhanga Districts. The magnitude of damage caused by this pest can be easily judged from the fact that it served as the chief cause of closure of the Seemapur factory in 1942. It is, therefore, a matter of importance to keep strict vigilance and check the spread of this pest by creating a buffer zone where no cane is cultivated as also to stop movement of cane not only from Bengal to Purnea but also from Purnea to the heavily concentrated cane districts of the Tirhut Division, where the burden of the sugar industry in Bihar lies. These strict domestic quarantine measures have helped to prevent its spread, but the cane crop in the district itself continues to suffer severely from its annual depredations. With the new factory coming up at Manihari in the district, need for controlling the pest has become paramount. Possibilities of utilising the larval parasite *Apanteles flavipes* in keeping the pest under control need to be explored. Further the egg parasite—*Trichogramma minutum* Riley has shown promising results and this together with the larval parasite should offer considerable relief.

XIII. Summary

1. *Chilo tumidicostalis* has been recorded as a serious pest of sugarcane in Purnea and adjoining parts of Bhagalpur, Monghyr and Darbhanga Districts.

2. Regular surveys were undertaken to study the distribution and intensity of this pest in Bihar. The incidence of the pest was studied on three bases, *viz.*, stalk, joint and length bored and relationship with edaphic and environmental conditions worked out besides also the relative resistance of varieties. Thus the pest was found to be more pronounced in heavy than light soils and under water-logged and flooded conditions than areas not subject to them. Similarly ratoons were more affected than the plant crop.

3. Of the varieties examined B.O. 10 was found to be most resistant and Co 421 the least. Varieties Co 513, Co 356 and B.O. 11 were more near B.O. 10 while Co 313, Co 331, Co 453 and Co 299 that of Co 421. It

appeared that solid-cored varieties were generally less affected compared to others that developed considerable pith and cavity.

4. In view of the gregarious habit of the borer the extent of damage caused both to cane tonnage and quality was considerable. Thus the loss in tonnage varied from 8·2–12·6% and that in sugar per cent. juice from 10·73–48·55%. Co 313 was the least damaged from this latter point of view.

5. The nature and characteristics of damage have been described in detail.

6. The biology of the pest has been studied under laboratory conditions and the different morphological characters have been described. The phenomenon of polymorphism has been observed to be well pronounced in the larvæ during the different seasonal conditions. The characters differentiating the pupæ of this borer from that of the other sugarcane moth borers have been detailed.

7. The biology of its larval parasite, *Apanteles flavipes* Cam., has also been studied under laboratory conditions. The marked difference observed in the case of antennæ of the male and the female parasite have been described and illustrated.

XIV. ACKNOWLEDGEMENTS

The work was carried out as a part of the sugarcane research scheme in Bihar being financed jointly by the Government of Bihar and the Indian Central Sugarcane Committee, to whom grateful thanks are due. Assistance rendered by the field staff of the Entomological Section and the computing staff of the Statistical Section is also acknowledged.

XV. REFERENCES

Cotes, E. C. .. *Indian Mus. Notes*, 1889, **1**, 22–29.

Fletcher, T. B. and Ghosh, C. C. *Proc. 3rd Ent. Mtg.*, Pusa, 1919, **1**, 388–89.

Hampson, G. F. .. *Ann. Mag. Nat. Hist.*, 1919, **3** (9), 437–57.

——— .. *Ibid.*, 1919, **3** (9), 53–68.

Issac, P. V. and Rao, K. V. .. *Indian Jour. Agric. Sci.*, 1941, **11** (5), 795–80.

——— and Venkatraman, T. V. *Ibid.*, 1941, **11** (5), 807–15.

Kapur, A. P. .. *Trans. R. Ent. Soc. London*, 1950, **101** (2), 389–434.

Khanna, K. L. and Bandyopadhyay, K. S. *Proc. Ind. Acad. Sci.*, 1951, **34** (4) **B**, 210–19.

Puri, V. D. .. *Curr. Sci.*, 1954, **23** (1), 21.

Trehan, K. N. and Butani, D. K. *Indian Jour. Agric. Sci.*, 1950, **20** (1), 79–85

Map showing incidence and distribution of *Chilo tumidicostalis*

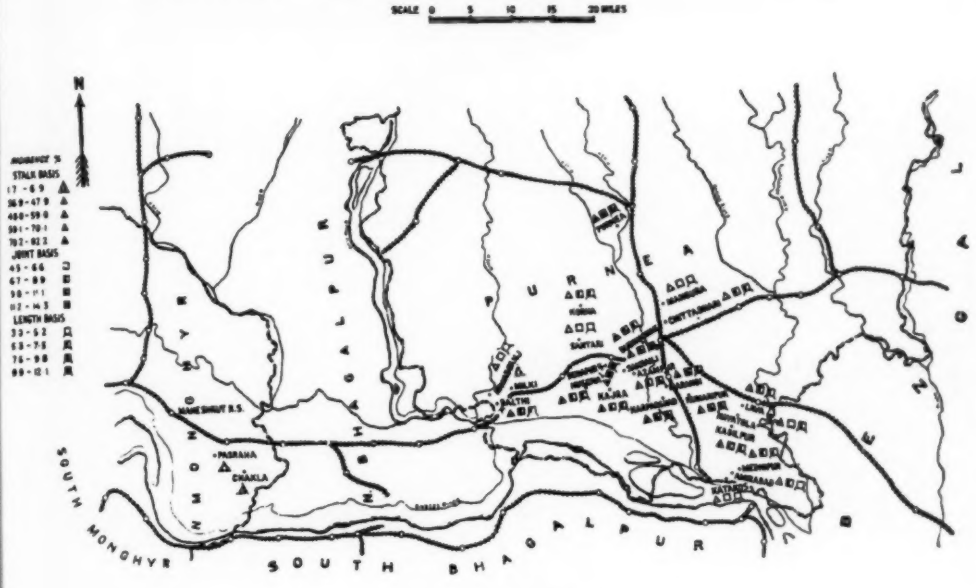


FIG. 1. North-East Bihar (1940).

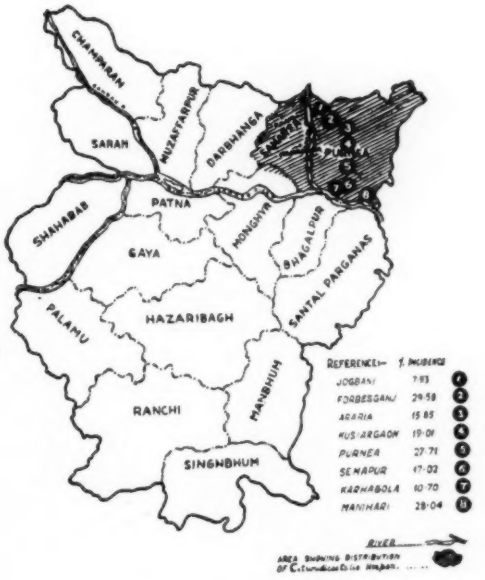
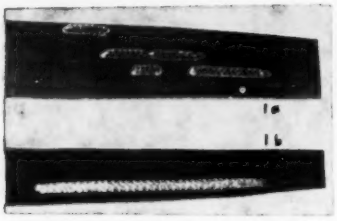


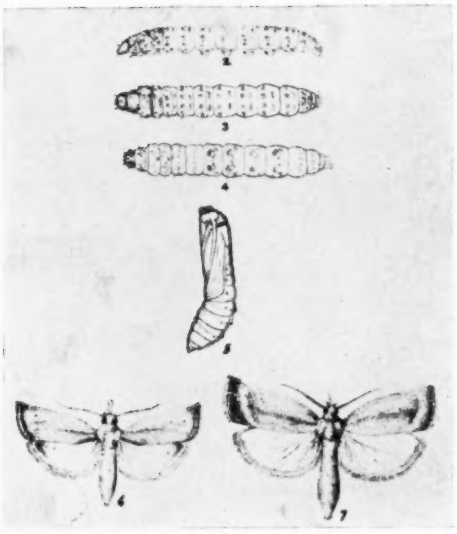
FIG. 2. Bihar (1953–54).

1

2

4

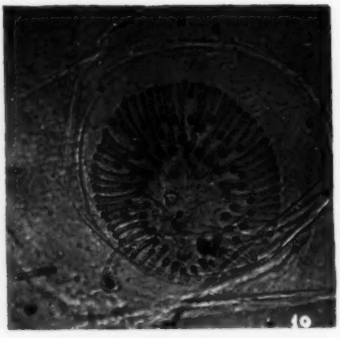

1a
1b


2
3
4
5
6
7

8

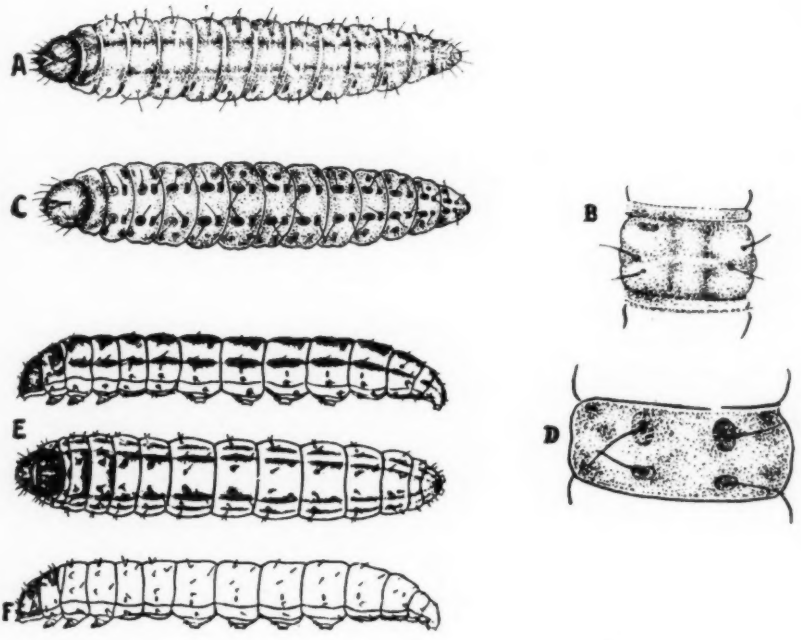
9a

9b


10

11

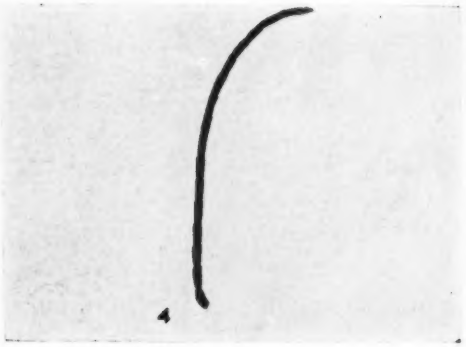
Chilo Tumidicostalis
(Polymorphism in Larvæ)

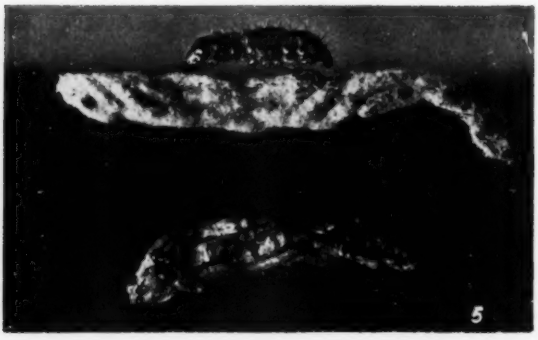


1

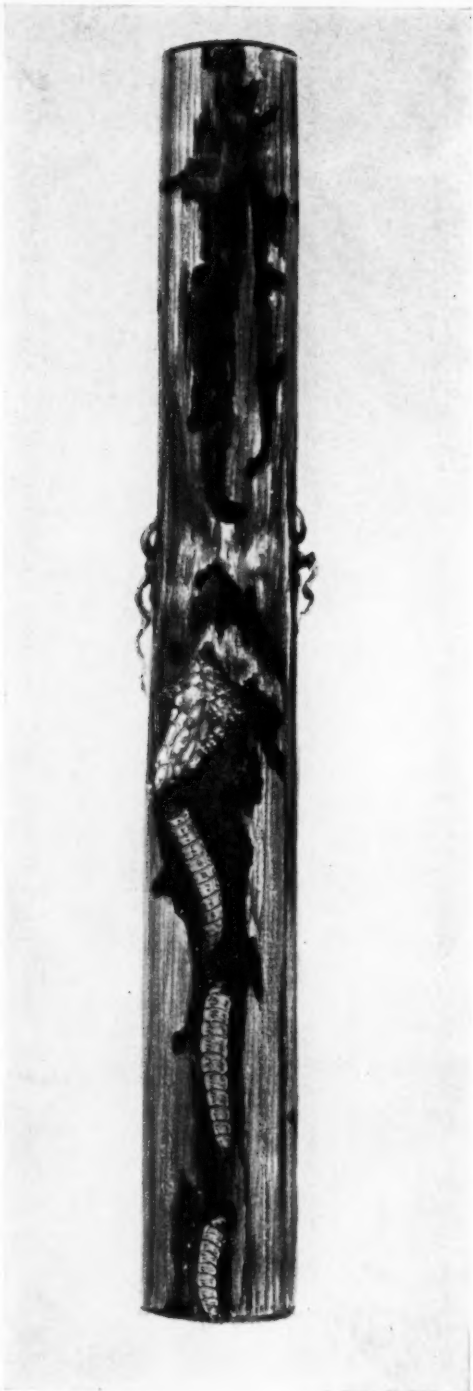
3

2


4


5

6



Explanation of Plates

Plate IV

Maps showing Incidence and Distribution of the Pest

Fig. 1. North-East Bihar (1940). Fig. 2. Bihar (1953–54).

Plate V

Damage Caused by Chilo tumidicostalis

Fig. 1. Cane completely hollowed by the borer has broken down by wind. It also shows the number of holes made by the borers. A piece is cut open longitudinally, showing the number of larvæ feeding inside.

Fig. 2. Various damaged canes split open longitudinally showing the ravages of the pest.

Figs. 3 and 4. Enlarged portions of the damaged cane, split open longitudinally, showing the typical symptoms of insect damage including the frass.

Plate V A

Cane stalk severely damaged by the borer showing numerous holes and also copious frass sticking to the stem as also deposited in between the stalk and leaf sheath.

Plate VI

Different Stages of Chilo tumidicostalis

Figs. 1 *a* and *b*. Egg masses.
Figs. 2, 3 and 4. Larvæ.
Fig. 5. Pupa (lateral view).
Fig. 6. Adult moth, male.
Fig. 7. Adult moth, female.
Fig. 8. Chætotoxy of larva.
Fig. 9 *a*. Female pupa (ventral view).
Fig. 9 *b*. Male pupa (ventral view).
Fig. 10. Crochets on the proleg of larva.
Fig. 11. Spiracle of larva.

Plate VI A

A=Larva showing Stripes.
B=Abdominal Segment showing Stripes.
C=Larva showing Warts.
D=Abdominal Segment showing Warts.
E=Larva showing Stripes and Warts Both.
F=Larva without Stripes or Warts.

Plate VII

Apanteles flavipes *Cam*., *A Larval Parasite of* C. tumidicostalis

Fig. 1. Adult female.
Fig. 2. Antenna of female.
Fig. 3. Adult male.
Fig. 4. Antenna of male.
Figs. 5 and 6. Cocoons of the parasite.

Plate VII A

Damaged cane piece split open longitudinally showing the white mass of cocoons of *Apanteles flavipes* lying in the larval tunnel near the host.

STUDIES ON THE ANATOMY OF THE SUGARCANE MOTH BORERS

III. Comparative Study of the Head and Its Appendages

By V. D. Puri, M.Sc. (Hons.), F.R.E.S.
(Central Sugarcane Research Station, Pusa, Bihar)

Received April 11, 1957
(Communicated by Shri K. L. Khanna, F.A.SC.)

CONTENTS

		Page
1.	Introduction	96
2.	Material and Methods	97
3.	External Features	97
4.	Sutures	98
5.	Sclerites and Areas of the Head Capsule	100
6.	Tentorium	102
7.	Appendages	
	(*a*) Compound Eyes	103
	(*b*) Mouth Parts	104
8.	Summary and Conclusions	106
9.	Acknowledgements	107
10.	References	108
11.	Explanation of Plates	108
12.	Key to the Lettering	109

1. Introduction

Anatomical studies of various moth borers of sugarcane have been taken up by quite a few workers in the past, but very little attention has been devoted to discuss and describe the same in detail, particularly on comparative basis. Gupta (1940) described the anatomy of *Chilotræa infuscatellus*, *C. auricilia*, *Proceras indicus* (*Diatræa venosata*) and *Chilo zonellus* but his descriptions are too brief and rather incomplete. Kapur (1950) described only the salient features of different species of moth borers associated with sugarcane belonging to *Crambinæ-Pyralidæ* with regard to their importance in their identification and classification. Krishnamurti and Usman (1952) have given a brief account of *Sesamia inferens* so far the anatomy is concerned,

Puri (1957) has described in detail the comparative morphology of the antennæ of *Scirpophaga nivella* (*Schœnobinæ-Pyralidæ*), *Chilo tumidicostalis* (*Crambinæ-Pyralidæ*), *C. zonellus*, *Chilotræa infuscatellus*, *C. auricilia*, *Bissetia steniellus* (*Chilo trypetes*), *Emmalocera depressella* (*Anerastiinæ-Pyralidæ*) and *Sesamia inferens* (*Agrotidæ-Noctuidæ*).

The comparative study of the head and its appendages in these eight species have been studied in detail and the same excluding the antennæ have been described in this paper, pointing out such of the characters that may be useful in their taxonomy and tracing out their phylogenetic relationships.

2. Material and Methods

The moths of *Scirpophaga nivella*, *Chilo tumidicostalis*, *C. zonellus*, *Chilotræa infuscatellus*, *C. auricilia*, *Emmalocera depressella* and *Sesamia inferens* were reared in the laboratory whereas the moths of *Bissetia steniellus* were received from the Cane Commissioner, Panjab, Entomologist to the Government of Panjab and Sugarcane Entomologist, Shahjahanpur.

The external study of the moth borers was carried on after soaking the specimens in 10% KOH. Permanent slides of the head capsules with different views and their different parts were prepared in the manner described by Puri (1954). Besides taking the direct microphotographs, the camera lucida drawings were also drawn where and when deemed necessary.

3. External Features

The cap-like head capsule of these insects is formed by the continuous sclerotization of the dorsal, anterior, lateral and posterior walls of the head. The head capsule in general, is comparatively a simple structure with a few sclerites which are modified in different species and prove useful in their identification. The most prominent of the anterior region is the frons, which shows great variations in its shape in different species, thus becoming a character of great taxonomic value in these insects. The frons is abruptly convex and may form a blunt or apical rounded or still apical pointed snout-like protuberance over the base of the mouth parts. Frontal sutures are present in the form of an arch in between the two antennal sockets as a double suture in all the species except in *Emmalocera depressella* where the frontal sutures are in the form of V (Plate XI, Fig. F) and indicates the primitiveness of the species. There are no traces of coronal or metopic suture in these species except in *E. depressella* and *Sesamia inferens* (Plate XII, Fig. B) where both the frontal as well coronal sutures are present. The coronal suture is feably developed in *E. depressella*. In *Sesamia inferens* the frontal arch touches the anterior rims of the antennal sockets while the coronal stem touches the

boundaries of the occipital foramen. In *Emmalocera depressella* the coronal suture does not seem to have any apparent connection with the frontal sutures and suggests that the frontal sutures or their ridges as these may be called, along with the stem of the so-called coronal suture may be the ecdysial cleavage lines of the head as suggested by Snodgrass (1947). This point is further confirmed by the study of these structures in *Sesamia inferens* where these structures appear as furrows and not as ridges. Fronto-clypeal suture separates the frons from the clypeus, which is a small distinct sclerite attached to the posterior margin of the labrum. A lateral pair of the compound eyes is situated on the dorso-lateral walls of the head while two ocelli are located on the vertex, below the bases of the antennæ. There is no trace of the median ocellus in any of the species, reported to be present in other insects. The foramen magnum or the occipital foramen is a small and almost V-shaped aperture with its arms divergent and widely located almost in the centre of the posterior surface of the head. The area of the posterior surface immediately surrounding the occipital foramen is distinctly depressed dorso-laterally. The posterior surface gradually levels up while approaching the eyes and the vertex. The oral fossa is located ventrally.

4. SUTURES

The following sutures may be evident in the head capsule of the different sugarcane moth borers. These sutures when evident form the lines of demarcation of the various component sclerites of the cranium. The sutures may be poorly developed or may form internal endoskeletal ridges as the case may be.

The epicranial suture.—It is almost suppressed in these species and does not exist in the form of a complete Y in any of the species under study. The frontal suture, as stated above, exists as an arched suture in between the bases of the two antennæ in all the species except in *E. depressella* and *Sesamia inferens* where the two frontal sutures exist in the form of V and an arched furrow in between th bases of the antennæ respectively. The later (Plate XII, Fig. B) is feably sclerotized indicating a depression like cleavage line. The coronal suture is completely obliterated in all the species under study except in *Emmalocera depressella* and *Sesamia inferens*. It is nothing but a poorly developed vertical stem in the former while in the latter it is a continuously produced posterior stem of the frontal sutures. Normally these sutures form the endoskeletal ridges but the present variations of three types, *viz*., absence of the coronal suture in most of the species, its presence in *E. depressella* as a discontinuous stem of the V-shaped frontal sutures and finally its existence as a continuous depression or furrow in *Sesamia inferen*

indicate that the epicranial suture which is composed of two frontal sutures and a coronal suture is not a permanent suture in the head capsule and probably represents the ecdysial cleavage lines of the head as suggested by Snodgrass (1947).

The occipital suture.—The occipital suture may be present in the dorsal posterior region of the head of these insects and is well developed as an internal horse-shoe-shaped ridge in *Chilo tumidicostalis* (Plate IX, Fig. G), crossing the back of the head and ending ventrally on each side of the epicranium before the posterior articulation of the mandibles. The internal ridge formed by the occipital suture serves probably to strengthen the posterior part of the epicranial walls. The position of this suture can be marked laterally in between the sub-genal sutures and the anterior tentorial arms.

Post-occipital suture.—It forms an internal post-occipital ridge which borders the foramen magnum dorsally and laterally. It widens dorsally into a semicircular lobe which projects outward over the occipital foramen while its ventral enlargements form the occipital condyles (Plate X, Fig. A) which serve as articulatory points for the lateral cervical sclerites. The anterior ends of the muscles moving the head are attached to the internal Post-Occipital Ridge.

The sub-genal sutures.—These sutures run laterally from below the posterior tentorial pits to the anterior articulation of the mandibles of each side and separate the Fronto-clypeal region from the sub-genal region which apparently merges on either side of the head with the triangular-shaped plates or lobes representing the rudimentary mandibles. It has been observed that this suture is well developed in the species where the tentorial pits lie in the sub-genal suture and is comparatively suppressed in *Emmalocera depressella* (Plate XI, Fig. I) where the anterior tentorial pits lie in the epistomal suture. In *Sesamia inferens* (Plate XII, Fig. A) also these sutures are not so prominent as in case of other sugarcane moth borers.

The epistomal or the fronto-clypeal suture.—This suture is very distinct and forms an epistomal ridge which forms a brace between the anterior mandibular articulations. This separates the broad, long frons from the clypeus which may be either a boat-shaped as in *Scirpophaga nivella* (Plate VIII, Fig. I), *Chilo tumidicostalis* (Plate IX, Fig. G), *Chilo zonellus* (Plate IX A, Fig. F), *Chilotræa infuscatellus* (Plate X, Figs. F and G), *Chilotræa auricilia* (Plate X A, Fig. C) and *Bissetia* steniellus (Plate XI A) or a transverse plate as in *Emmalocera depressella* (Plate XI, Fig. I) on the antero-ventral side of the head. Laterally it touches the two sub-genal sutures of both the sides.

Ocular sutures.—These sutures are represented by the grooves which surround the compound eyes, each forming internally an endoskeletal ridge around the eye.

Antennal sutures.—Each antenna is set in an antennal socket, the rim of which is reinforced by a sub-marginal ridge. The external groove of this may be called the antennal suture. These touch the inner margins of the compound eyes laterally and demark the narrow antennal sclerites which border the antennal sockets.

Post-genal sutures.—The endoskeletal ridges formed by these sutures separate the post-genæ from the post-occiput.

5. Sclerites and Areas of the Head Capsule

The following sclerites of the cranium have been observed and studied:—

The frons.—The term frons has generally been used in an indefinite sense for the facial area of the head above the clypeus by the entomologists in the past but is actually a part of the post-oral anterior wall of the cranium as described by Snodgrass (1947). It may be separated out from the clypeus by the epistomal suture. It extends dorsally upto the frontal sutures in between the antennal bases and becomes a median unpaired sclerite of the facial area of the head capsule lying in between the ocular sutures of the lateral compound eyes. The term frontal or frontoclypeal area has been suggested instead of frontoclypeus by him. The term frontoclypeal area may be used in the cases where the epistomal suture is not well developed to separate the two structures the frons and the clypeus. Frons is greatly modified in these insects and is of great diagnostic value in their identification. In *Scirpophaga nivella* (Plate VIII, Figs. A, F and H) it is convex, the bulging portion being either projected forward with a blunt apical point or rounded. In *Chilo tumidicostalis* it is abruptly convex with a distinct inverted V-shaped corneous point near its apex (Plate IX, Fig. G). In *C. zonellus* the apex of the frons is in the form of a conical projection or snout bulging forward like the rhinal extension (Plate IX A, Figs. A and E), but the apical point is rather thick and strong. In *Bissetia steniellus* the frons is projected forward more vertically with a sharp pointed apex like the point of a needle (Plate XI A, Figs. A, B and D). It is widely rounded, abruptly convex, produced forward and is flattened dorso-ventrally in *Chilotræa infuscatellus* (Plate X, Fig. G). In *Chilotræa auricilia* it is convex with its antero-lateral sides sub-rounded and produced forward with a central apical point sharply raised and pointed at its convex level (Plate X A, Figs. A and E). In *Emmalocera depressella* the frontoclypeal area is sub-rounded, convex and comparatively wider and less produced forward (Plate XI, Fig. F). It is abruptly convex, compara-

ively narrow than that of *Chilotræa infuscatellus* and produced forward without any apical point in *Sesamia inferens* (Plate XII, Fig. B).

The clypeus.—The clypeus is a pre-oral sclerite attached to the basal part of the labrum. It has been observed to be a boat-shaped independent sclerite in most of the species. It does not form an independent boat-shaped sclerite in *Emmalocera depressella* as explained above and where the anterior tentorial pits lie in the epistomal suture instead of sub-genal ones and the arms of V-shaped frontal suture lie in between the antennæ anteriorly (Plate XI, Fig. F). It has been observed to be in the form of a transverse horizontal plate in case of *Scirpophaga nivella* (Plate VIII, Fig. I).

The vertex.—It is a large convex area of the head constituting the top of the head capsule. It is not limited by any special suture. Two lateral ocelli are located on the vertex and are present in all the species under study.

Sub-genal areas.—The sub-genal areas in all these species apparently seem to merge on either side of the head with the triangular and rudimentary mandible which are separated from the frons and the clypeus by the sub-genal sutures. These areas are very narrow and sometimes covered over by the area of the epistomal suture when these are to provide for the anterior tentorial pits as in case of *Emmalocera depressella.*

The genæ.—The area of each genæ is a narrow, elongated space lying in between the occipital suture and the posterior half of the ocular sutures. Dorsally it extends upto the vertex while ventrally it is demarcated by the pleurostomal suture, *i.e.*, the part of the sub-genal suture above the mandibles.

The narrow bands encircling the compound eyes within the ocular sutures are known as ocular sclerites while the marginal areas of the antennal sockets defined by the antennal sutures are termed the antennal sclerites.

The occipital arch.—It is the horse-shoe-shaped band between the occipital and post-occipital sutures on the posterior surface of the head as in case of *Chilo tumidicostalis* (Plate IX, Fig. G). The dorsal part of this arch is known as the occiput while the lateral parts of this arch are known as the post-genæ or the post-genal areas, which lie posterior to the genæ. Since the occipital may not be very distinct or may appear to be even absent these areas may be called as the posterior region of the head.

The post-occiput.—The narrow posterior rim of the epicranium set off from the occipital arch by the post-occipital suture to which is also attached the neck membrane is called the post-occiput. The ventro-lateral posterior margins of the post-occiput are produced on each side in a small condylar process the occipital condyle to which is articulated peg-like projection of

the anterior arm of the lateral cervical sclerite of the neck. The post-occipital ridge is also very prominent endo-skeletal ridge to which are attached the muscles. The post-occipital region may appear as an incomplete ring or sclerite called the post-occipital sclerite which borders the occipital foramen dorsally and laterally. It serves as a buffer area between the foramen and the occipital arch. It widens dorsally into a semi-circular lobe which projects outwards over the foramen.

The occipital foramen.—The occipital foramen is an aperture with its divergent arms in the form of a V and is situated almost in the centre of the posterior region of the head in all these insects.

Post-genæ.—The post-genal areas as stated above are the spaces between the post-occiput and the genæ. They are separated from the post-occiput by the post-genal sutures.

Ocelli.—The two ocelli are located on the vertex just below the base of each antenna and are very prominent structures.

6. TENTORIUM

The tentorium in all these insects is a well developed structure and can be studied best either in the macerated specimens or whole mounts of the head capsules. In *Scirpophaga nivella* (Plate VIII, Figs. A, F and H), *Chilo tumidicostalis* (Plate IX, Fig. G), *C. zonellus* (Plate IX A, Figs. A and E), *Chilotræa infuscatellus* (Plate X, Figs. A and G), *C. auricilia* (Plate X A, Fig. A) and *Bissetia steniellus* (Plate XI A, Figs. A and B) it is of a similar primitive type and consists of a pair of anterior tentorial arms arising from the anterior tentorial pits or fossæ which lie in the sub-genal sutures above the bases of the mandibles or above the anterior mandibular articulation. Each anterior tentorial arm extends forward to the sides of the clypeus ventrally and then backwards dorsally through the interior of the head capsule, to meet the posterior tentorial arms of the tentorium forming the transverse posterior tentorial bridge across and above the occipital foramen. The posterior tentorial arms of the tentorium arise from the posterior tentorial pits and constitute the above mentioned transverse bar over and above the foramen magnum or the occipital foramen, when seen through the back of the head. The dorsal arms are very feebly developed and run anteriorly towards the epistomal suture and posteriorly towards the bases of the antennæ. These seem to be of secondary origin as outgrowths of the tentorial structure and provide for the attachment of the antennal muscles. It has been observed that such type of tentorium is well developed in species whose head capsule is of hollow

and globose type and not dorso-ventrally flattened or flat like the one found in *Emmalocera depressella* (Plate XI, Fig. F) where it is very poorly developed. In that case it consists of almost the similar plan of structures mentioned above with a variation in the position of the anterior and posterior tentorial pits the former of which are contained in the epistomal suture suggesting a primitive character in evolution while the posterior tentorial pits are comparatively distantly located laterally thus increasing the length of the transverse bar or the posterior tentorial bridge. In *Sesamia inferens* (Plate XII, Figs. A and B) it is somewhat different from the type already described above in case of Pyralid moth borers, as it suggests a modified tentorium in Lepidoptera. It consists of an anterior median plate or central circular plate between the bases of the maxillary plates, which is formed by the two median arms or processes which arise from the roots of the anterior tentorial arms. This median plate may be homologous to the so-called corporotentorium described by Snodgrass (1935). These processes are also united in an anterior bridge before the circum œsophageal connectives, which seem to pass through this median plate. The anterior tentorial arms and the feably developed dorsal arms arise from the anterior tentorial pits. The anterior tentorial arms are very prominent with their broad, rounded and club-shaped bases supported comparatively on thin roots or arms. The dorsal arms run towards the epistomal suture anteriorly. The posterior portions of these dorsal arms are very feebly developed and run towards the bases of the antennæ. It again seems that these are the secondary outgrowths of the tentorial structure to provide for the attachment of the antennal muscles. The anterior tentorial pits are not so well marked and are correspondingly elongate (Plate XII, Fig. C) and not rounded as in case of *Pyralidæ*, mentioned above. Because of the club-shaped and flattened anterior tentorial arms, the anterior median plate and the posterior arched tentorial bridge, the area of the head capsule in between these structures appears to be somewhat dumble-shaped. The posterior tentorial arms as usual arise from the posterior tentorial pits situated a little above the external margins of the posterior tentorial arms and form the transverse posterior tentorial bridge across and above the occipital foramen.

7. Appendages

(*a*) *Compound Eyes*

These are sub-globular, prominent and very large structures situated laterad to the head capsule. Their greatest development is ventro-posteriorly and they project beyond the ventral and posterior regions of the head capsule. The inner margins are curved slightly and are approximated ventrally to some extent. Narrow ocular sclerites separate the eyes from the frons and the

clypeus or the so-called frontoclypeal areas. Dorsally these sclerites apparently terminate at the antennal sockets and ventrally they appear to merge with the subgenal regions of the head capsule. The development of the eyes has completely obliterated the genæ, in these insects.

(*b*) *Mouth Parts*

The labrum.—(Plate VIII, Fig. B; Plate IX, Fig. A; and Plate IX A, Fig. A) consists of a narrow transverse membranous sclerite, situated on the ventral side, attached to the clypeus. It is produced ventrad into a mesal and two lateral projected pilifers bearing long bristles. The pilifers are present in all the species in addition to the triangular vestigeal plates representing the rudimentary mandibles which can hardly be of any functional use. The mesal lobe constitutes the epipharynx in part, but seems to be much depressed and reduced in comparison to the pilifers which exhibit various modifications of shape and structure in different species under study. These modifications have been observed to be valuable in the identification of different species to some extent. Pradhan and Aren (1941) described the labrum of *Scirpophaga nivella* as a narrow membranous sheet provided with lancet-shaped processes or the pilifiers, one each on either side of its lateral side bearing a large number of bristles. These observations agree with those of the present author. These are lancet-shaped structures in *Scirpophaga nivella* (Plate VIII, Fig. E). In *Chilo tumidicostalis* and *C. zonellus* (Plate IX, Fig. F) and (Plate IX A, Fig. D) these structures are comparatively much reduced and less prominent. In *Chilotræa infuscatellus* (Plate X, Fig. E) these structures are little bigger lobe-like projections of the labrum as also are in *Chilotræa auricilia* (Plate X A, Fig. D). In *Bissetia steniellus* (Plate XI A, Fig. C) these are comparatively more developed and are club-shaped prominent structures. In *Sesamia inferens* (Plate XII, Fig. C) these are still more prominent and flattened structures. In *Emmalocera depressella* (Plate XI, Fig. E) these appear to be minute structures which are somewhat turnip-shaped.

Mandibles.—The mandibles are present but are nonfunctional in all these species. These are rudimentary triangular plates situated on either side or behind the pilifers. Pradhan and Aren (*loc. cit.*) have described these to be typical in case of *Scirpophaga nivella* which are provided with bases bearing slight condylar thickening on its inner edges and a long tendon attached to the outer angle of each. These are somewhat longer in size in *Chilotræa auricilia* (Plate X A, Fig. C) and *Chilotræa infuscatellus* (Plate X, Fig. F). These are still bigger in *Emmalocera depressella* (Plate XI, Fig. I) and *Bissetia steniellus* (Plate XI, Figs. B and C). These are reduced in *Sesamia inferens* (Plate XII, Fig. A). There is no point of articulation with the head capsule

and there are no definite sutures demarcating the mandibles from the sub-genæ. However the vestiges of such sutures may be detected in case of most of these species.

Maxillæ.—The maxillæ are the largest and most important of the mouth parts in these insects and one can easily study the various parts even without dissecting the specimens by preparing the whole mounts.

The stipes and the cardo are quite clear and prominent in all the species. The sub-cylindrical stipes is generally observed to curve mesally and is almost completely concealed beneath the projecting portion of the sub-gena. It articulates laterally with the head capsule and is thus situated ventro-posteriorly to the vestigeal or the rudimentary mandibles. The proximal end of the stipes articulates with a small triangular cardo provided with a basal condyle to articulate with the cranium. Cardo proper is comparatively much reduced and is shorter than the area of the basal condyle. The cardo also seems to articulate posteriorly with the sclerotized portions of the labium.

The maxillary palpi in general, are somewhat palmate in shape, four-segmented, greatly reduced in size, and are borne on the anterior portion of the stipes near the point of attachment to the galea. An interesting type of reduction in their size, shape and number of segments may be observed sometimes in case of *Emmalocera depressella* (Plate XI, Figs. C, H and I) where these appear to be either three-segmented or four-segmented with the second distal segment modified into a rounded lobe-like structure. In *Sesamia inferens* (Plate XII, Figs. A, B and C) the maxillæ are completely obliterated and are absent. The entire surface of these structures, when or where present, is covered with a large number of longitudinally fluted scales of varying length.

The distal end of each stipes bears a lateral piece or galea which together constitute the so-called the galeæ or the proboscis which form the greater part of the maxillæ in these insects. The two galeæ are held together by interlocking grooves or ridges (Plate XII, Fig. C). The proboscis remain coiled when not in use as shown in *Bissetia steniellus* (Plate XI A, Fig. C). The opposed walls of galeæ are thick, concave and enclose between them a food canal or channel through which the liquid food or the drink is sucked up by the sucking pump into which opens the basal portion of the food canal, as illustrated in case of *Chilotræa auricilia* (Plate X A, Fig. F) and *Emmalocera depressella* (Plate XI, Fig. G) in the horizontal view of the tip of the organ. The galeæ of *Scirpophaga nivella* have been studied in detail by Pradhan and Aren (*loc. cit.*) and their observations hold true for all the eight

species but for the lacinia which is comparatively poorly developed and one can easily presume their absence in these species.

Labium.—The structure of the labium is rather indefinite and the sclerotized portions are limited to two small areas around the base of each labial palpus, and to a short narrow median strip proximad of the base of the proboscis. The remainder probably is represented by the membranous portion of the ventral surface of the head.

The labial palpi are three-segmented in these insects and may be sometimes quite helpful in separating the different species. The basal segment of each labial palpus is well developed and is curved forward and upward in such a way so that the remaining segments are applied closely to the anterior surface of the head. The second segment is the longest in case of all the species. In *Scirpophaga nivella* (Plate VIII, Figs. A and C) it is about 1½ times to twice the length of the third or the distal segment which is without the sensory pit, a character which separates this particular species from the rest of the species. In *Chilo tumidicostalis* (Plate IX, Figs. A and D) and *C. zonellus* (Plate IX A, Figs. A and C) the second segment is about 5–7 times the length of the distal segment, as also is the case in *Chilotræa infuscatellus* (Plate X, Figs. A and C) and *C. auricilia* (Plate X A, Figs. B and C). The distal segment of *Chilo* species is comparatively longer than those of *Chilotræa* species. In *Bissetia steniellus* (Plate XI A, Fig. D) the second segment is 4–5 times the length of the distal segment. In *Emmalocera depressella* (Plate XI, Figs. D and I) it is only 3–4 times the length of the distal segment and in *Sesamia inferens* (Plate XII, Fig. A) it is about twice the length of the distal segment of the palpus. The distal segment of all the species are provided with a ventral sensory pit lined with short hairs near the apex except in *Scirpophaga nivella* as explained above. These ventral pits when present seem to represent a sensory organ. The entire surface of the labial palpi is covered with long hairs.

8. SUMMARY AND CONCLUSIONS

1. A detailed anatomical description of the head and its appendages of eight different species of sugarcane moth borers, *viz.*, *Scirpophaga nivella*, *Chilo tumidicostalis*, *C. zonellus*, *Chilotræa infuscatellus*, *C. auricilia*, *Bissetia steniellus*, *Emmalocera depressella* and *Sesamia inferens* has been worked out and described on comparative basis.

2. The position of the epicranial suture is not constant and the various modifications of the frontal sutures are quite interesting and have been described in detail. It is quite probable that these represent the ecdysial cleavage lines in these insects.

3. The occipital suture does not form an internal horse-shoe-shaped prominent ridge in most of the cases but it is very prominent in case of *Chilo tumidicostalis* where it crosses the back of the head and ends ventrally on each side of the epicranium before the posterior articulation of the mandibles as illustrated.

4. The frons exhibits various modifications and has been observed to be an important character in the identification of the different species.

5. The clypeus which is almost a boat-shaped structure does not possess the boat-shaped structure if the anterior tentorial pits lie in the epistomal suture instead of lying in the sub-genal area as observed and described in case of *Emmalocera depressella*.

6. Two modifications of the tentorial structures have been observed in different families *Pyralidæ* and the *Noctuidæ* which are of special interest and have been discussed and described in detail.

7. The mouth parts which are of piercing and sucking type have been studied and their various structures described in detail. The shape of the pilifers seem to be quite helpful in separating certain species from one another.

8. The maxillary palpi which are generally four-segmented are palmate show a tendency towards reduction in size as well as in number of segments and may sometimes be observed to possess only three segments with the second distal segment modified into a rounded lobe-like structure as in case of *Emmalocera depressella*. The maxillary palpi are completely absent in *Sesamia inferens*.

9. The structure of the labium is quite indefinite in all the species and the distal segment of the labial palpi is provided with a ventral sensory pit near their apices except in *Scirpophaga nivella*, a character which separates this particular species from other species.

9. Acknowledgements

This work forms a part of the Scheme of Sugarcane Research Fellowships donated by the Sugar Industry of Bihar and the author is indebted to the donors for the facilities thus created to carry out an investigation of this nature. His grateful thanks are also due to Shri K. L. Khanna, Director, Sugarcane Research and Development, Bihar, Pusa, for suggesting the problem and guiding it besides going through the manuscript. He is also thankful to Shri D. K. Butani, Field Entomologist, for helpful suggestions in writing of the manuscript. The help rendered by Shri G. S. Lall Nainjee and Shri R. P. Shrivastava, Photographers of the Station, is also acknowledged.

10. REFERENCES

Gupta, B. D. .. *Ind. J. Agric. Sci.*, 1940, **10**, 787–817.

Kapur, A. P. .. *Proc. R. ent. Soc. Lond.*, 1950, **101** (11), 389–434.

Krishnamurti, B. and Usman, S. *Bull.* 15, *Dept. Agri., Mysore*, Entomological Series, 1952, pp. 72.

Pradhan, S. and Aren, N. S. .. *Indian J. Ent.*, 1941, **3** (2), 177–95.

Puri, V. D. .. *Curr. Sci.*, 1954, **23**, 21.

———— .. *Proc. Ind. Acad. Sci.*, 1957, **45**, 32–42.

Snodgrass, R. E. .. *Principles of Insect Morphology*, McGraw-Hill Book Company, Inc., New York, 1935, pp. 667.

———— .. *Smithsonian Misc. Collect.*, 1947, **107** (7), 52.

11. EXPLANATION OF PLATES

PLATE VIII. *Scirpophaga nivella*

(A) Head (ventral view).
(B) Mouth parts and anterior tentorial pits.
(C) Labial palpus.
(D) Maxillary palpus with cardo and stipes.
(E) Pilifer.
(F) Head (Dorsal view).
(G) Antennal socket and ocelli on vertex.
(H) Head (Dorsal view).
(I) Head (Ventral view).
(J) Head (Side view).

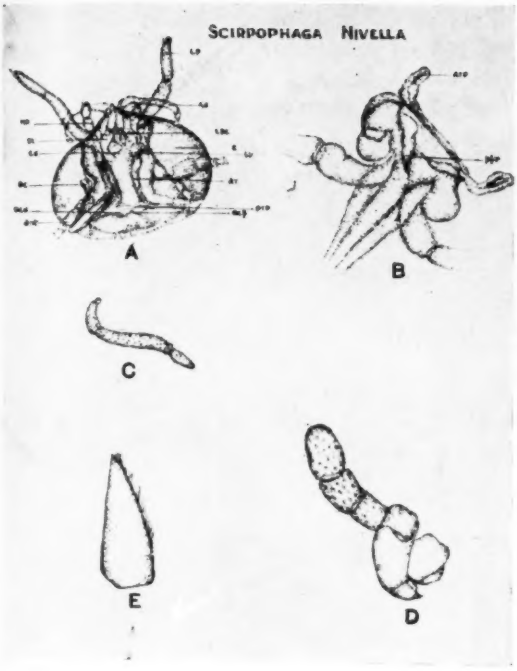
PLATE IX

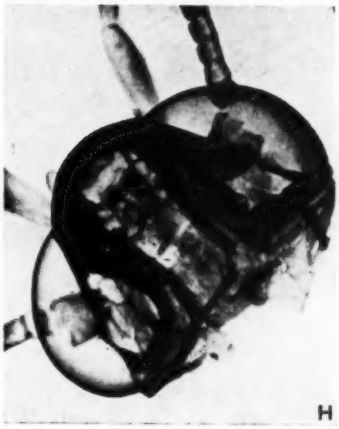
IX. *Chilo tumidicostalis*

(A) Head (Dorsal View).
(B) Head (Ventral view).
(C) Anterior tentorial pits.
(D) Labial palpus.
(E) Maxillary palpus.
(F) Pilifer.
(G) Head capsule (Dorsal view).
(H) Head capsule (Ventral view).
(I) Corneous apical point of the frons.

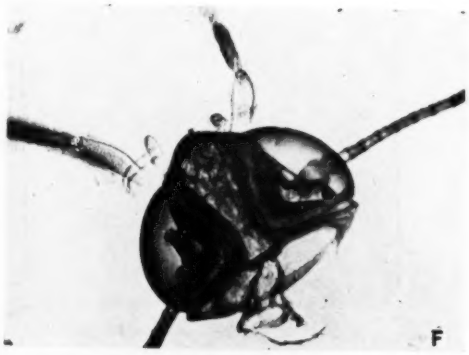
IX A. *Chilo zonellus*

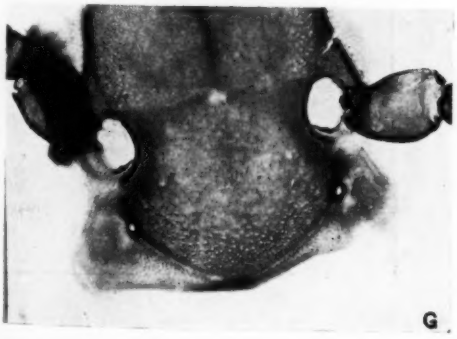
(A) Head capsule (Ventral view).
(B) Maxillary palpus.
(C) Labial palpus.
(D) Pilifer.
(E) Head capsule (Dorsal).
(F) Head capsule (Ventral).


SCIRPOPHAGA NIVELLA
A
B
C
D
E


H

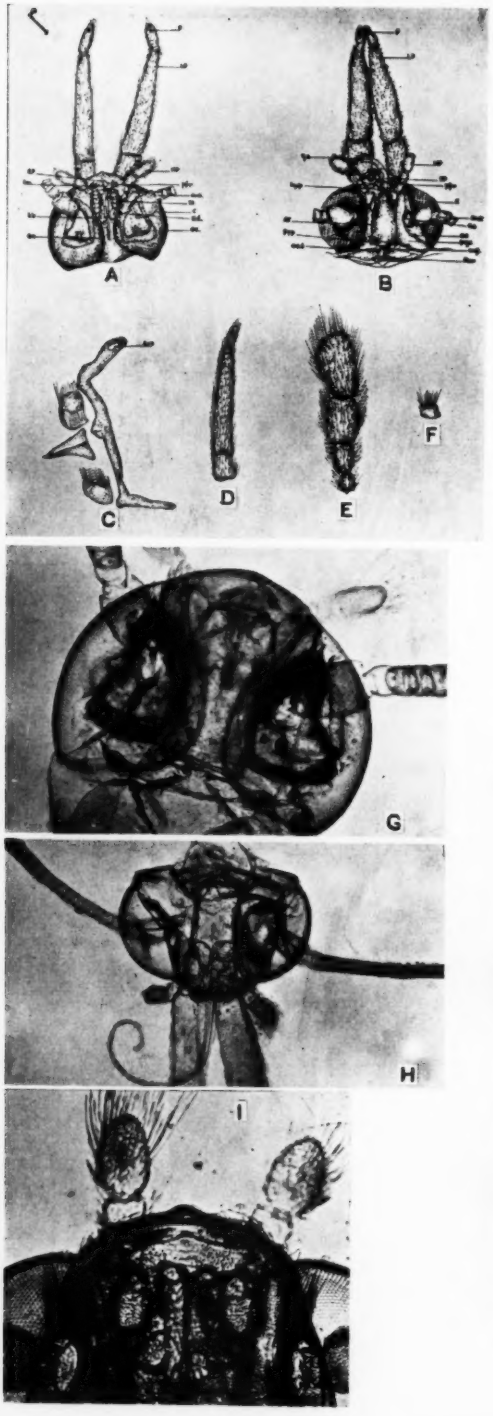
I


F


G

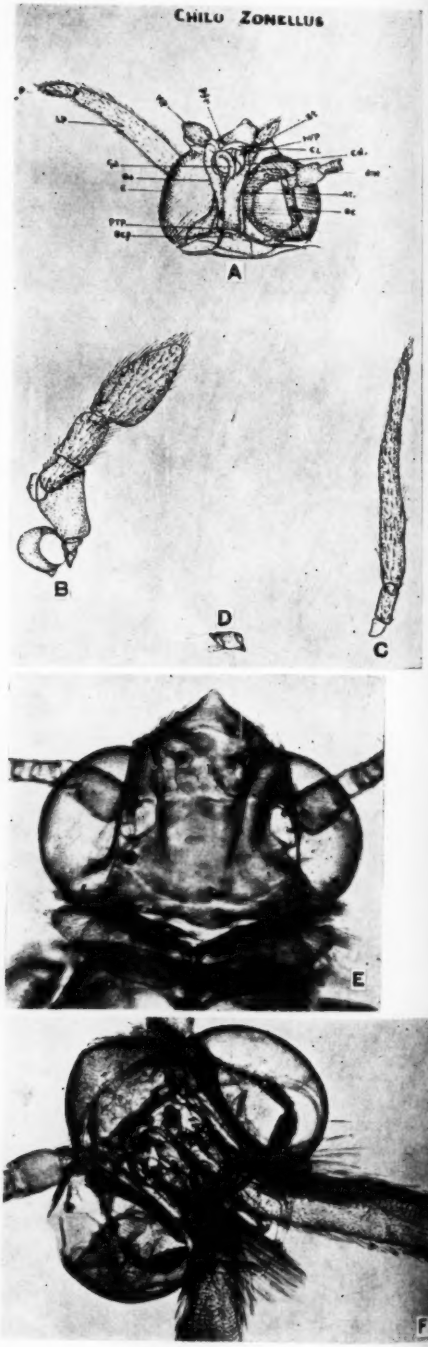
J

CHILO TUMIDICOSTALIS



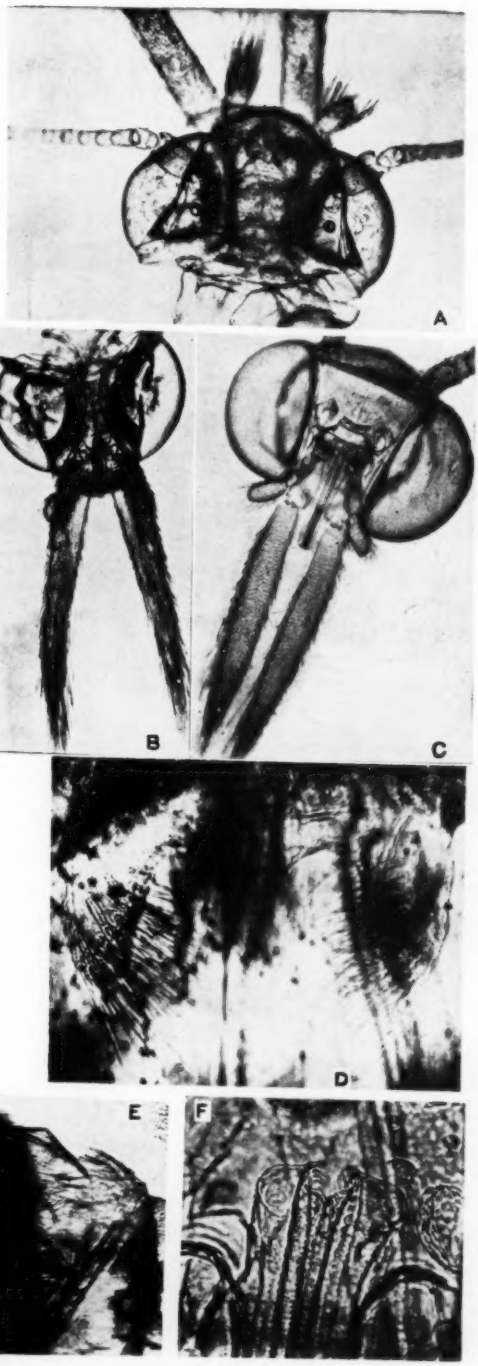
IX

CHILO ZONELLUS

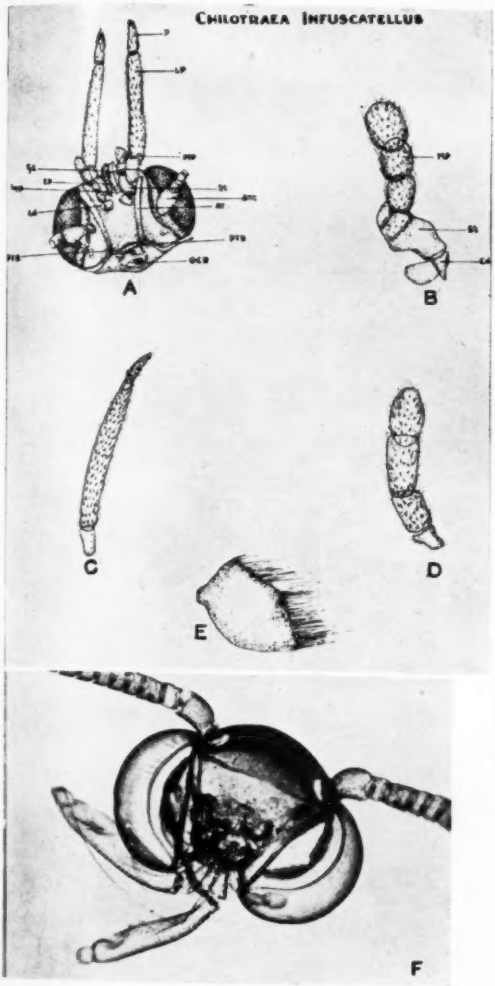


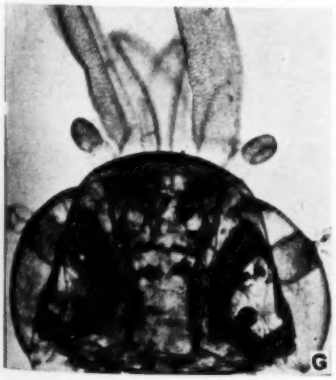
IX A

CHILOTRAEA AURICILIA



CHILOTRAEA INFUSCATELLUS

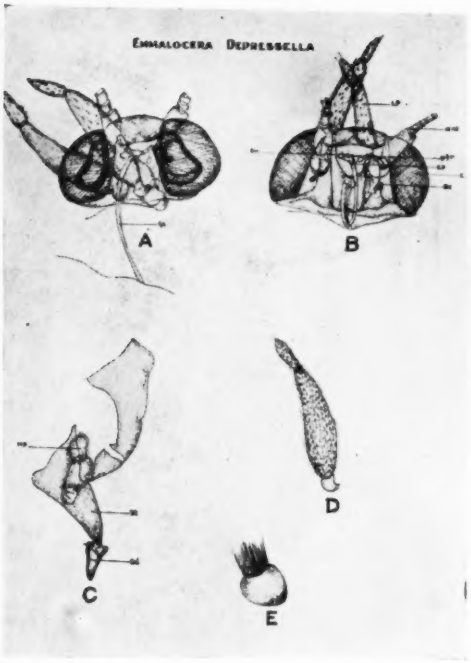


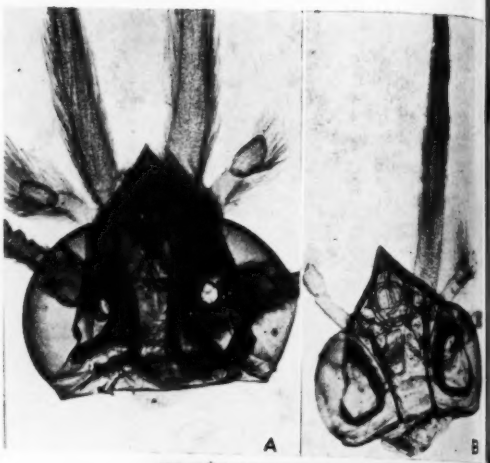


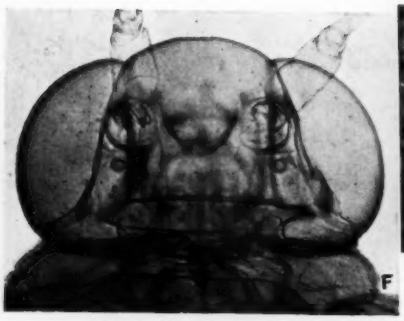
X

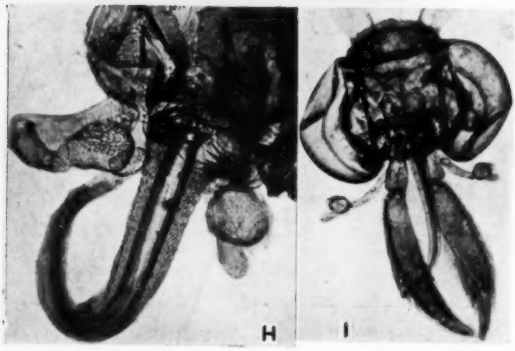
X A

BISSETIA STENIELLUS



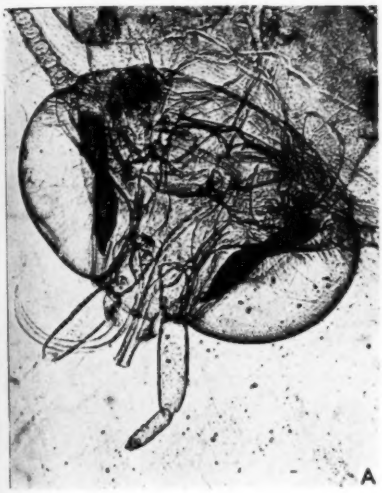






XI XI A

SESAMIA INFERENS



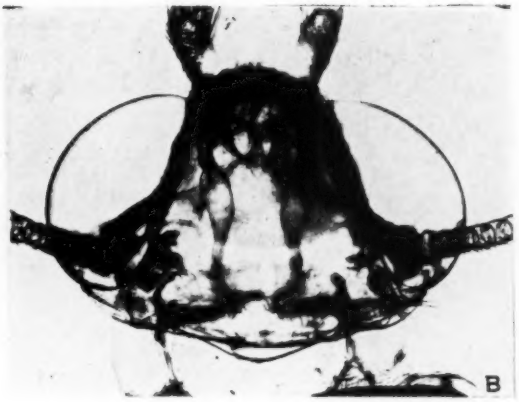


PLATE X

X. *Chilotræa infuscatellus*

(A) Head capsule (Ventral view).
(B) Maxillary palpus with cardo and stipes.
(C) Labial palpus.
(D) Maxillary palpus.
(E) Pilifer.
(F) Head capsule and mouth parts (Ventral view).
(G) Head capsule (Dorsal view).

X A. *Chilotræa auricilia*

(A) Head capsule (Dorsal view).
(B) Head capsule (Ventral view).
(C) Head capsule (Ventral view).
(D) Pilifer.
(E) Apical point of the frons.
(F) Tip of the proboscis (Horizontal view).

PLATE XI

XI. *Emmalocera depressella*

(A) Head capsule (Latero-ventral view).
(B) Head capsule (Antero-ventral view).
(C) Maxillary palpus with cardo and stipes.
(D) Labial palpus.
(E) Pilifer.
(F) Head capsule (Dorsal view).
(G) Tip of the proboscis (Horizontal view).
(H) Mouth parts and the pilifers.
(I) Head capsule and mouth parts.

XI A. *Bissetia steniellus*

(A) Head capsule (Dorsal view).
(B) Head capsule (Ventral view).
(C) Coiled proboscis and the pilifers.
(D) Head capsule and the labial palpi.

PLATE XII. *Sesamia inferens*

(A) Head capsule (Ventral view).
(B) Head capsule (Dorsal view).
(C) Portion of the proboscis and pilifers.

12. KEY TO THE LETTERING

ant., antenna; *AT.*, Anterior tentorial arm; *ATP.*, Anterior tentorial pit; *Cd.*, Cardo; *CL.*, Clypeus; *E.*, Compound eye; *EP.*, Epipharynx; *ga.*, Galea; *hyp.*, Hypopharynx; *Lb.*, labium; *lm.*, Labrum; *LP.*, Labial palpus; *MP.*, Maxillary palpus; *Oc.*, Ocellus; *Ocd.*, Occipital condyle; *Ocf.*, Occipital foramen; *pfr.*, Pilifer; *PTP.*, Posterior tentorial pit; *St.*, Stipes.

PHOSPHORUS CYCLE IN THE SEA WITH PARTICULAR REFERENCE TO TROPICAL INSHORE WATERS

By R. Jayaraman and G. Seshappa

(*Central Marine Fisheries Research Sub-Station, Bombay*)

Received April 20, 1957

(Communicated by Dr. N. K. Panikkar, F.A.Sc.)

CONTENTS

		Page
I.	Abstract	110
II.	Introduction	111
III.	Review of Literature	
	(*a*) Temperate and Cold Waters	111
	(*b*) Tropical Waters	115
IV.	Observations on Total Phosphorus Content ..	117
V.	General Remarks	120
VI.	Summary	123
VII.	References	124

I. Abstract

A brief account is given about the distribution and cycle of phosphates in the sea in the temperate and tropical waters, the recent investigations in the Indian waters being discussed in some detail. The data on the total phosphorus content of the waters along the Indian coast show that the values for total P are generally more than 3 times and sometimes nearly 40 times the values given by Harvey for the waters of Western English Channel. A hypothesis is presented which would explain the observed cycles of the phosphate–phytoplankton relationships on the Indian coastal waters to some extent, particularly in the Malabar waters. While the monsoon high values are caused by the release of locked-up phosphates in the bottom, it is suggested that a continuous source of replenishment is essentially to be presumed for the long duration of the simultaneous maxima of both phosphates and phytoplankton during the monsoon. Three such sources of replenishment are indicated. The post-monsoon 'erratic' fluctuations in phosphates and phytoplankton can be explained as due to a somewhat cyclical repetition of the usual phenomena of utilization, regeneration and phytoplankton production, until low levels of both phytoplankton and phosphates are established during the subsequent pre-monsoon months.

II. Introduction

An important problem that has for sometime been engaging the attention of marine biologists all over the world is the phosphate cycle. Our present knowledge of this subject is mostly derived from the investigations in the temperate and colder waters. The most intensive work in this direction has been done by the workers in the Marine Biological Laboratory at Plymouth, the standardisation of the methods for the estimation of minute concentrations of phosphates in sea-water by Atkins being actually the beginning of the studies on the phosphate in sea. On the American continent a very large amount of work has been done in recent years at various institutions, such as the Woods Hole Oceanographic Institution, Scripps Institution of Oceanography, California, and the Oceanographical Laboratories of the University of Washington, Seattle. In the Southern hemisphere, some work has been done in Australia (Dakin and Colefax, 1935, Rochford, 1951). A certain amount of work seems to have been done by the Japanese workers, but most of their work has been published in the Japanese language and translations of the same are not easily available.

As a result of all these investigations to date a good deal of knowledge has been gained on the distribution and the cycle of phosphates in the sea and their role in the fertility of the coastal and offshore waters. This knowledge is, however, still very far from complete.

III. Review of Literature

(*a*) *Temperate and cold waters.*—In 1923 Atkins developed the method for phosphate estimation (" the Atkins-Deniges method ") and applied it to the study of the variation of phosphate in a single station in the English Channel. Subsequently the distribution of phosphates have been studied in the three oceans not only by several individual workers in isolated localities but also by expeditions which have worked a number of stations (Sverdrup *et al.*, 1942). The observations have, however, been more or less scattered in nature. But these have given some idea about the horizontal and vertical distribution of phosphate in the open ocean.

Phosphorus exists in the sea in inorganic as well as organic forms and in solution as well as particulate form. This forms an important constituent of all living matter which must collect it from its environment. With the destruction of living matter, it must return to the environment in some form or the other. In sea-water phosphorus is present mainly as phosphate-ions whose distribution is markedly affected by organic agencies. Phosphate-phosphorus has in fact been found to be one of the substances that may limit

production of plant life. While much is known about the dissolved inorganic phosphate in sea-water our knowledge is still scanty concerning the amounts of phosphorus present as particulate or dissolved organic phosphorus.

The largest volume of literature is available on investigations in localized areas, the object of these investigations being to get a fairly complete picture of the seasonal cycle of changes in phosphate and also to obtain an idea of the important controlling factors. The seasonal variations of phosphate at a position in the English Channel has been followed at monthly intervals for a number of years by the workers in the Plymouth Laboratory (Harvey, 1955). Dakin and Colefax (1935) have studied the seasonal variations in the phosphate in the waters off New South Wales, Australia. A study of the seasonal variations over a five-year period in the surface waters of the San Juan Channel, Friday Harbour, Pacific Coast, has been made by Phifer and Thompson (1937). Several other workers have studied similar seasonal variations in the phosphate content along with planktological and other marine biological studies. One main thesis that has been brought out as a result of all these investigations is that there is a regular seasonal cycle of phosphate in the temperate and colder waters from year to year. Highest concentrations are found in winter and lowest in spring and early summer. The low concentrations in the summer months are attributed to the outburst of phytoplankton populations which utilise the phosphate for growth and multiplication.

In the course of the studies on the seasonal cycles of phosphates in the different regions it was found necessary to know about the sources of availability of the phosphates and how the concentration in the sea is regulated so as to produce well-defined seasonal cycles. As the study of the seasonal cycles have, in most cases, been restricted to the coastal waters—both from the point of view of convenience of observations as well as from the necessity to exploit these waters for the fishery wealth—the first and most important hypothesis put forward was that relating to the importance of rivers and other forms of land drainage in contributing to the phosphate content of the neritic waters (Harvey, 1927, p. 174; Sverdrup *et al.*, 1942, p. 213). But Riley's (1937) work on the influence of the Missisipi river drainage on the phosphate content of the Northern waters of the Gulf of Mexico has shown that river waters do not contribute appreciably to the phosphate content of the Gulf waters. Recently, Graham, Amison and Marvin (1954) in their studies on the phosphorus content of waters along the West Coast of Florida have also shown that the rivers on this coast opening out into the Gulf do not contribute in any measurable degree to the phosphate content of the waters of the Gulf. One of the main reasons as stated by Harvey (1955) is that in general,

river waters do not have a high phosphate content—hardly exceeding 0·16–0·32 μg. at. P/L, unless heavily polluted.

The importance of bottom deposits as a source of phosphates has been stressed by a number of workers. In certain shallow areas phosphatic concretions with high phosphorus concentrations have been found. The mode of origin of these concretions is not known (Sverdrup *et al.*, 1942). Only in recent years attempts have been made to study the mechanism of exchange of phosphates between the bottom deposits and the overlying column of water. Among these may be mentioned the works of Hutchinson (1941) in Linseley Pond, Connecticut, Mortimer (1941 and 1942) in English Lakes, Miller (1952) on Biscayne Key, Florida and the work of Rittenberg, Emery and Orr (1955) in the basins off the Californian coast. Rochford (1951) as a result of his work on the Australian estuarine waters has provided a considerable volume of data on the phosphates and has given an indication of the nature of changes taking place in the bottom muds leading to the release of phosphates to the overlying water. Attempts have been made by Stephenson (1949) and Carritt and Goodgal (1954) to study, under controlled laboratory conditions, the mechanisms involved in the release of phosphates from the muds. The interchange of phosphate between the offshore sea floor and the water above has also been indicated by Cooper (1951) during his studies on the chemical properties of sea-water in the neighbourhood of the Labadie Bank. Evidence of regeneration from the bottom was seen in this case where the bottom was muddy and none where it was otherwise.

The biological aspect in the cycle of phosphates may be briefly summarised as the utilisation of phosphates by the phytoplankton and subsequent regeneration, probably through bacterial activity from the dead and decaying matter. A good summary of the biological factors involved in the phosphate cycle in the sea has been given by Harvey (1955). Harvey has stated that

(i) Some species of phytoplankton can absorb phosphate as quickly as they need it for rapid growth when its concentration exceeds a threshold value whose magnitude lies in the region of 0·52 μg.-at. P/L;

(ii) They can continue absorption of phosphate and its conversion into organic phosphorus compounds throughout day and night; and

(iii) They can build up a reserve of storage product which cannot be used directly for further synthesis without prior dephosphorylation and that light sets free or activates the phosphorylase concerned.

The role of bacteria in the phosphate cycle in the sea has been worked out by Renn (1937) and Waksman and Renn (1936). The same has been explained by the following hypothesis by Stephenson (1949) with reference to his experimental work on aerated estuarine mud samples and sea-water:

"(i) When mud and sea-water are mixed by stirring, or when muddy sea-water is filtered, or when the filtrates are agitated, bacteria are destroyed;

(ii) Their protoplasm, rich in phosphorus, is broken down by living bacteria, with release of phosphate;

(iii) The bacteria grow, using the remaining organic matter, now poor in phosphorus; and

(iv) During growth, they reabsorb the phosphate previously released."

In a study of the cycle of phosphorus in the Western basin of the North Atlantic, Seiwell (1935), Seiwell and Seiwell (1938) have shown that the sinking of the decomposing plankton in sea-water results in oxygen consump-

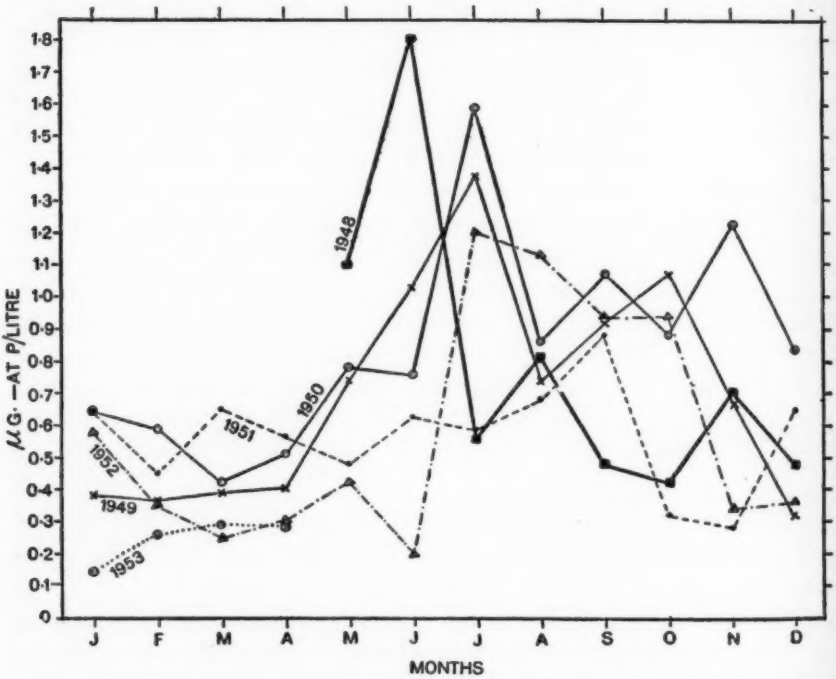


FIG. 1. Showing the mean monthly values for phosphates in the surface waters off the Malabar Coast during the years, 1948 to 1953.

tion and phosphate liberation. Cooper (1935) and Waksman, Hotchkiss, Carey and Hardman (1938) have also shown that bacteria liberate phosphate from dead zooplankton added to sea-water.

(*b*) *Tropical waters.*—The amount of published work on the subject of the phosphate cycle in tropical waters is very small in comparison with the publications on the temperate waters. Relatively speaking, systematic hydrological investigations in the tropical waters are more or less of recent origin which explains the smaller number of publications from this region. Most of the work done so far relates to inshore waters. Among the earliest publications may be mentioned that of Orr (1928–29) on the waters in the neighbourhood of the Great Barrier Reef, off the North-East Australian coast. Delsman's paper on the plankton in the Java Sea (1939) includes a short discussion on the phosphate cycle in those waters. Tham Ah Kow (1953) has worked on the physical, chemical and biological conditions of the waters of the Singapore straits in which he has included studies on the phosphate content also. Some work has been done in the coastal waters around Zanzibar by Newell (Private communication) but the same does not appear to have been published so far. Investigations on the chemistry of the Caribbean and Cayman seas by Rakestraw and Smith (1937) and on the Venezuela Bay by Redfield (1955) have given us some idea of the distribution of phosphates in the tropical waters in the Western hemisphere. During recent years the work of the Pacific Oceanic Fishery investigations group in the equatorial and tropical areas of Pacific Ocean (1954) have furnished some data on the trends in the distribution of phosphates in these grounds. The reports published by them were intended more to present a tabulated data of the various oceanographic factors including phosphates and dissolved oxygen. The phosphate content in the surface layers in most of the stations worked by them ranged between 0·25 and 0·82 μg.-at. P/L. Values as low as 0·06 μg.-at. P/L have been encountered in a few instances. A relatively higher concentration of phosphates about the equator, in the surface layer, has been shown to be an evidence for equatorial upwelling.

Investigations in the Indian waters have been restricted to localized areas along the coast and the earliest paper giving values for phosphate content is that of Chidambaram and Menon (1945) for the Malabar Coast. The paper by Bal *et al.* (1946) gives a systematic account of the physico-chemical conditions of the waters of the Arabian Sea near the Bombay Harbour during the year 1944–45. The seasonal distribution of phosphates has been given along with other factors for this area. The scope of these data is restricted as the station in which the samplings were done was very much influenced

by the waters from inside the harbour and a certain amount of pollution is indicated.

The investigations by Jayaraman (1951) on the waters of the Bay of Bengal represents the earliest attempt to understand the distribution and seasonal cycle of nutrients—particularly phosphates—on the east coast of India. The studies were initiated as a preliminary to understanding the fundamental problem of the productivity of the tropical inshore waters. Observations were carried out over a period of 17 months and this author has been able to observe seasonal variations somewhat similar to those observed in the temperate waters. Very high phosphate values have been observed in the months, May–September, the range of values being 0·5–3·0 μg.-at. P/L the highest value being in the month of August. October–March seems to be the period of phosphate minima in this area, the lowest value being in November. This work has also shown an inverse correlation between phosphates and dissolved oxygen in these waters.

The observations in this region were continued by Ramamurthi (1953) who found that in 1951–52, the seasonal differences were not as marked as before. The range of phosphate values in this case was 0·3–1·2 μg.-at. P/L the maximum being in May and minimum in February. Ramamurthi has also recorded the vertical distribution of the dissolved phosphate at 12 fathoms.

In the southern section of the east coast, studies were made by Jayaraman on the waters of the Gulf of Mannar and Palk Bay near Mandapam (1954) and by Chacko, Valsan and Pillai (1954) on the waters of the Kundugal Channel of the Gulf of Mannar. The mean monthly values for phosphates obtained by Jayaraman in the vicinity of Mandapam varied between 0·15 and 0·30 μg.-at. P/L in the Gulf of Mannar and between 0·12 and 0·25 μg.- at. P/L in Palk Bay. The phosphate content of these waters is, in general, low and also there does not appear to be any marked seasonal cycle. There is no instance, however, when these waters have been completely exhausted of phosphates. Chacko, Valsan and Pillai (1954) and Chidambaram, Rajendran and Valsan (1951) have obtained somewhat similar values for the waters of the Gulf of Mannar in the stations different from those worked by Jayaraman. In the Kundugal Channel, Chacko *et al.* have indicated the possibility of the waters deriving the nutrients from the land source.

On the Malabar Coast detailed investigations on the hydrology and plankton of the inshore waters have been carried out by George (1953). He has given a discussion of the phosphate cycle in those waters and has correlated the same with the occurrence and seasonal cycle of plankton. He has

observed very high phosphates in the waters after the onset of the monsoons and attributes it to the churning of the mud bottom during this stormy period. He also suggests that the mountain streams opening out in the neighbourhood might be exerting some influence. The occurrence of high values during the monsoon period has been observed by him in all the years of his study. In the post-monsoon months, George has observed variations in phosphate content differing from year to year. George has also observed that the bottom samples of water invariably showed a high value throughout the year and found that the differences were more striking during the monsoon months and during the period of mud bank formation. The normal range of phosphate values, according to George, was 0·35–0·56 μg.-at. P/L which increases substantially during the monsoon. The occurrence of high phosphate values has been shown to be a regular feature and it is interesting to note that there was a swarming of diatoms in all these months. The usual phenomenon of depletion of phosphates during the period of diatom outburst observed in temperate waters has not been noticed by this author in the monsoon period. In the post-monsoon months, however, he has observed variations in phosphate content corresponding to the periodic outbursts of diatoms. Thus the variations in phosphate, according to George, has shown different trends in monsoon and non-monsoon months. Panikkar and Jayaraman (1953) have suggested that besides the local turbulence, the northerly transport of the waters (during S.-W. Monsoon) which have upwelled near the Maldive-Laccadive ridge might be one of the factors contributing to the high phosphate content of the waters of the Malabar Coast.

A detailed account of the role of bottom muds in the phosphate cycle of Malabar Coast has been given by Seshappa (1953) and Seshappa and Jayaraman (1956). The factors involved in the retention and release of phosphates from the mud have been examined in these papers. It has been found that there are differences in the nature and composition of the muds between monsoon and non-monsoon months. High moisture and silt content and high concentration of phosphates in the interstices of the muds were the most important features during the pre-monsoon months. From the pre-monsoon to the monsoon months, there is a sudden reduction in the values for all these factors with a high increase of phosphates in the overlying column of water.

IV. Observations on Total Phosphorus Content

The importance of the total phosphorus estimations (combined organic and inorganic) in a study of phosphate cycles in different waters has been indicated by Redfield *et al.* (1937), Armstrong and Harvey (1950) and

Ketchum *et al.* (1955). Harvey (1950) has stated that total phosphorus values in the waters help to distinguish one water mass from another and the phosphorus content is a measure of its potential fertility having a relation to annual production of vegetation and zooplankton. Soule *et al.* (cited by Ketchum and others 1955) have also used total phosphate values to identify different water masses in the North Atlantic. In reviewing the work on the phosphate cycle in Indian waters, it seemed useful to include a short account of our preliminary studies in the distribution of total phosphorus in the waters along the Indian Coast. The data included in this section have not been published before and are based on analyses of samples collected from 42 stations, in each of the periods July–August and in January–February—the former, representing the period of South-West Monsoon and the latter, North-East Monsoon. Table I shows the values for total P in the coastal waters in the different latitudes. The determination of the total P was done according to the procedure described by Rochford (1951). The method used for deriving the average values for each latitude has been described by Jayaraman and Gogate (1956, under publication). An examination of the total P values of the waters of the west and east coasts reveals the following features:—

In the Period, July–August

1. The total P values along the different latitudes vary between 2·1–12·8 μg.-at. P/L on the West Coast and 1·7–3·0 μg.-at. P/L on the East Coast.

2. On the West Coast, the values are higher north of 13° N. Latitude than south of it.

3. On the East Coast, the highest value for total P is seen in 19° N. In general, the distribution of total P is more uniform on the East Coast than on the West Coast.

In the Period, January–February

1. The range of values from latitude to latitude is from 1·1–5·5 μg.- at. P/L on the West Coast and from 1·1–4·7 μg.- at. P/L on the East Coast.

2. Highest value is found in 22° N. on West Coast and in 15° N. on the East Coast.

3. Between 19° and 22° N. the values are much higher on the West Coast.

4. South of 18° N. the values are nearly of the same order of magnitude barring a few deviations.

Suryanarayana Rao (Private communication) has also obtained similar high values from an inshore station near Calicut. In both the periods, there

TABLE I

Distribution of salinity and total phosphorus in the coastal waters around India

Period	N. Lat.	West Coast (Arabian Sea)			East Coast (Bay of Bengal)		
		Salinity S‰	Total phosphorus		Salinity S‰	Total phosphorus	
			μg. P/L	μg.-at. P/L		μg. P/L	μg.-at. P/L
July-	22	..	..	..	..	..	..
August	21	..	..	..	31·46	56	1·8
1952 (S.-W.	20	..	..	..	26·53	53	1·7
Monsoon)	19	..	..	..	25·82	93	3·0
	18	36·53	99	3·2	30·66	74	2·4
	17	36·18	105	3·4	32·68	71	2·3
	16	36·35	99	3·2	33·21	71	2·3
	15	35·55	87	2·8	33·82	65	2·1
	14	36·00	198	6·4	34·43	62	2·0
	13	34·61	397	12·8	33·91	68	2·2
	12	..	..	..	..	..	..
	11	32·50	81	2·6	34·09	71	2·3
	10	33·57	81	2·6	33·57	81	2·6
	9	34·09	65	2·1	33·73	62	2·0
	8	34·96	81	2·6	33·73	71	2·3

Period	N. Lat.	West Coast (Arabian Sea)			East Coast (Bay of Bengal)		
		Salinity S‰	Total phosphorus		Salinity S‰	Total phosphorus	
			μg. P/L	μg.-at. P/L		μg. P/L	μg.-at. P/L
January-	22	36·33	171	5·5	..	..	..
February	21	..	71	2·3	30·62	56	1·8
1953	20	..	71	2·3	29·92	56	1·8
(Close of	19	35·24	90	2·9	33·61	50	1·6
N.-E.	18	35·71	62	2·0	33·60	68	2·2
Monsoon)	17	35·88	37	1·2	34·67	34	1·1
	16	34·32	71	2·3	33·90	81	2·6
	15	33·96	50	1·6	34·67	146	4·7
	14	33·44	50	1·6	34·58	68	2·2
	13	33·26	78	2·5	34·31	56	1·8
	12	33·78	34	1·1	34·14	40	1·3
	11	..	..	..	34·14	43	1·4
	10	33·61	53	1·7	33·44	59	1·9
	9	33·44	43	1·4	35·19	43	1·4
	8	33·35	62	2·0	35·53	37	1·2

is no correlation between the values for total P and salinity. In other words, as stated by Armstrong and Harvey (1950), the geographical distribution of total P does not coincide with the geographical distribution of salinity in the present instance also.

Armstrong and Harvey (1950) have stated that the total P in Western English Channel is as little as 0·32 μg.-at. P/L while in other areas it is double or more than double. The present set of values are more than 3 times and sometimes nearly 40 times this value. Kalle (cited by Armstrong and Harvey, 1950) has shown that in the turbid waters of the southern North Sea a large quantity of phosphorus is present as detritus—giving values as high as 1·3–1·6 μg.-at. P/L at some positions.

V. GENERAL REMARKS

The following important points have been brought out in regard to the distribution of phosphates in the tropical waters as a result of the recent investigations in the Indian waters. It should, however, be mentioned that these should not be taken as representing truly the conditions in the more open waters of the tropical regions.

In most of the typical temperate waters (*e.g.*, English Channel) the maximum values for phosphates in the surface waters has been below 0·7 μg.-at. P/L. Much higher values have, however, been reported in some exceptional instances such as the waters of the San Juan Channel (Phifer and Thompson, 1937). In the waters along the Indian Coast—both in the West Coast and in some areas on the East Coast—the upper limit of values have been found to be much higher than in the temperate waters. The maximum values for the phosphate content of the waters of Malabar Coast have been shown to be 2·94 μg.-at. P/L (George, 1953) and 3·00 μg.-at. P/L for the waters off the Madras City—East Coast (Jayaraman, 1951). The mean monthly values fluctuate between 0·10 and 1·80 μg.-at. P/L in the Malabar waters and between 0·30 and 1·20 μg.-at. P/L in the Madras waters. In the waters near Mandapam, however, the range is 0·15–0·26 μg.-at. P/L. It could thus be seen that the phosphate concentrations of the surface waters along the Indian Coast are much higher or nearly of the same order of magnitude as those of the typically temperate waters.

It is also to be mentioned that as in the temperate waters, the phosphate values show distinct seasonal trends, more particularly in the waters along the Malabar Coast. The seasons in our waters are, however, closely related to the prevalence of the monsoons, especially the South-West Monsoon along the West Coast of India. Along the East Coast, the Madras waters have shown a marked seasonal cycle of phosphates during one year but the

same has not been repeated in a subsequent year. The phosphate content of the waters near Mandapam has, however, not shown any marked seasonal trend. It is, therefore, observed that although the actual concentrations of phosphates have been high in a number of areas, marked seasonal cycles have been consistently obtained only in the waters along the Malabar Coast. From the point of view of marine biology, the knowledge of marked seasonal cycles of phosphates, as well as other nutrients is of great interest as it would help in the prediction of seasons of high and low organic production.

The most important correlation that has been so far established between the biology of the sea and its phosphate content is in relation to the production of phytoplankton which, in its turn, limits the quality and quantity of the animal life in any marine area. An inverse relationship between phytoplankton and phosphate content has been observed to be the rule in temperate waters. In the Malabar waters, George (1953) observed that phytoplankton peak was during the monsoon months, but the phosphates also showed peak values during all these months; following the monsoon months, there were minor peaks of phosphates and also phytoplankton. Prasad (1954) states that relationships other than mere utilization and production must be involved in the phosphate cycle in the waters near Mandapam. He also states (1956), "It is often believed that the normal relationship between plankton and nutrients is inverse, although the converse has also been observed (Riley, 1941). This assumption of inverse relationship is based primarily on the observations made in temperate areas, and very little information is available on the nature of relationship that exists between plankton and the various nutrient salts in the tropical areas." Some light is thrown on this problem by the intensive investigations at Calicut (George, 1953; Seshappa, 1953 and Seshappa and Jayaraman, 1956).

The facts at present known about the phosphate levels and the biological cycle in the inshore waters at Calicut (Malabar Coast) are as follows:—

1. During the South-West Monsoon (June–August) both phosphates and phytoplankton are very high; at the commencement of the monsoon there is a heavy mortality of the bottom animals and the sea is rough throughout the period.

2. During the post-monsoon months, the sea is calm. Resettlement of bottom animals takes place, and there are minor peaks of both phytoplankton and phosphate values, the fluctuations being "erratic".

3. During the following pre-monsoon months, the sea is calm and both the p hytoplankton and phosphate values are low. The bottom deposits

contain high quantities of adsorbed and interstitial phosphates, the latter being low during the monsoon and show " erratic " fluctuations during the post-monsoon months.

Seshappa and Jayaraman (1956) have suggested that the monsoon increase of phosphates is due to the agitation of the bottom deposits releasing the interstitial phosphates; the sources of phosphate in the deposits are mentioned to be the decaying material resulting from the large-scale mortality of bottom animals and also the laterite silt brought in by land drainage.

The fertilization experiments in the Scottish lochs (Marshall and Orr, 1948) have shown that when large quantities of phosphates are added to sea-water and other conditions are suitable, phytoplankton blooms follow within a few days of fertilization, the phosphate values falling steeply within a couple of weeks and the plankton values also coming down a little later. This loss of phosphates is due partly to utilization by phytoplankton and partly to adsorption and interstitial retention in the bottom deposits. Hayes and Coffin (1951) added radioactive phosphorus to lakes with the objective of studying the cycle of added phosphate. They found that the photosynthetic activity of the phytoplankton removed only a part of it. Precipitation as ferric phosphate according to them might have removed another part, sorption on to suspended solids, a third portion and lastly lateral diffusion and eddy mixing might have effected further reduction of phosphate. These authors have stated that all these factors are of importance and the relative contribution of each of these factors is dependent, to a large extent, on local conditions.

The plankton bloom in Malabar Coastal waters continues practically throughout the monsoon season and if the phosphate increases in the water column during this period were only limited to what has accumulated up to the commencement of the monsoon as a result of the local turnover, it would appear likely that the quantities thus released would be insufficient to cause the continuous high phosphate values in the waters during the monsoon months and also to sustain such a high level of plankton production. Additional and extraneous sources must, therefore, be presumed. Evidence has already been mentioned about two such sources, namely land drainage and mortality of bottom animals. A third source of replenishment is possible in the onshore transport of the upwelled water near the Maldive-Laccadive Ridge (Panikkar and Jayaraman, 1953). It seems obvious, in any case, that unless a continued replenishment of the phosphates is available, the local accumulation by itself would be insufficient to sustain high phytoplankton and phosphates for such a long period.

The various " erratic " relationships noticed by George (1953) can be explained by the following assumptions which appear to be reasonable on the basis of the literature reviewed in this paper.

1. In general, a high phosphate production is followed by a high phytoplankton production, the ascending curves for the P and phytoplankton being parallel to each other until optimum levels are reached.

2. If there is no continued replenishment of the phosphate the phytoplankton maximum is of a short duration; otherwise it is of a longer duration.

3. When the phytoplankton maximum is reached and there is no further replenishment of the phosphate, utilization will exceed availability and phosphate will show a downward trend resulting in the well-known normal reciprocal relationship of low phosphate and high phytoplankton.

4. This stage is eventually followed by decrease in phytoplankton as a result of grazing by zooplankton and also death.

5. Regeneration *in situ* of phosphate following (4) due to liberation of the phosphatases during the decay of the plant cells.

6. Repetition of phytoplankton bloom, phosphate utilization, etc., as above, subject to other conditions being suitable.

7. Return of stable conditions at the sea bottom and establishment of a low level of phytoplankton production along with low levels of phosphates; locking up of phosphates in the bottom deposits.

8. Release of the nutrients from the sea bottom and initiation of the annual cycle.

In Fig. 1 are shown the monthly mean values of the phosphates in the surface waters of the Malabar Coast from 1948–53. The trends shown in the different years appear to fit in well with the above hypothesis.

VI. Summary

1. A brief account is given about the distribution and cycle of phosphates in the sea in the temperate and tropical waters, the recent investigations in the Indian waters being discussed in some detail.

2. Some original observations on the total phosphorus content of the waters along the Indian Coast from 84 stations covering two seasons of the year are also presented. The values for total phosphorus are seen to be more than 3 times and sometimes nearly 40 times those given by Armstrong and Harvey (1950) for the Western English Channel.

3. A hypothesis is presented which would explain the observed cycles of the phosphate–phytoplankton relationships on the Indian Coast to some extent, particularly in the Malabar waters. While the monsoon high values

of phosphates are caused by the release of locked-up phosphates in the bottom, it is suggested that a continuous source of replenishment is essentially to be presumed for the long duration of the simultaneous maxima of both the phytoplankton and phosphates during the monsoon. Three such sources of replenishment are indicated. The post-monsoon 'erratic' fluctuations in phosphates and phytoplankton can be explained as due to a somewhat cyclical repetition of the usual phenomena of utilization, regeneration and phytoplankton production, until low levels of phosphates and phytoplankton are established during the subsequent pre-monsoon months.

VII. REFERENCES

1. Armstrong, F. A. J. and Harvey, H. W. — *J. Mar. Biol. Assn. U.K.*, 1950, **29**, 145–62.
2. Bal, D. V., Pradhan, L. B. and Gupte, K. G. — *Proc. Ind. Acad. Sci.*, 1946, **24 B** (2), 60–74.
3. Carritt, D. E. and Goodgal, S. — *Deep Sea Res.*, 1954, **1**, 224–43.
4. Chacko, P. I., Valsan, A. P. and Pillai, C. M. — *Contr. from Mar. Fish. Biol. Stn., Krusadai*, Govt. Press, Madras, 1954, pp. 1–32.
5. Chidambaram, K. and Menon, M. D. — *Proc. Ind. Acad. Sci.*, 1945, **27 B**, 355–67.
6. ———, Rajendran, A. D. I. and Valsan, A. P. — *Jour. Madras Univ.*, 1951, **21**, 48–74.
7. Cooper, L. H. N. .. — *J. Mar. Biol. Assn. U.K.*, 1933, **18**, 161.
8. ———— .. — *Ibid.*, 1935, **20**, 197.
9. ———— .. — *Ibid.*, 1938, **23**, 181.
10. ———— .. — *Ibid.*, 1951, **30**, 21.
11. Dakin, W. J. and Colefax, A. N. — *Proc. Linn. Soc. N.S.W.*, 1935, **60**, 303.
12. Delsman, H. C. .. — *Treubia*, 1939, **17** (2), 139–82.
13. George, P. C. .. — *J. Zool. Soc. India*, 1953, **5** (1), 76–107.
14. Graham, H. W., Amison, J. M. and Marvin, K. T. — *Spl. Sci. Report—Fisheries No.* 122—U.S. Department of Interior, Fish and Wild Life Service, Washington, 1954, 1–43.
15. Harvey, H. W. .. — *Biological Chemistry and Physics of Sea Water*, Cambridge Univ. Press, 1927.
16. ———— .. — *J. Mar. Biol. Assn., U.K.*, 1950, **29**, 97.
17. ———— .. — *The Chemistry and Fertility of Sea-Waters*, Cambridge Univ. Press, 1955, 224 pp.
18. Hayes, F. R. and Coffin, C. C. — "Radioactive phosphorus and exchange of Lake nutrients," *Endeavour*, 1951, **10**, 78–81.
19. Hutchinson, G. E. .. — *Ecol. Monogr.*, 1941, **11**, 21–60.
20. Jayaraman, R. .. — *Proc. Ind. Acad. Sci.*, 1951, **33 B**, 92–99.
21. ———— .. — *Ind. J. Fish.*, 1954, **1**, 345–64.
22. ——— and Gogate, S. S. .. — "Salinity and Temperature variations in the waters of the Arabian Sea off the Bombay and Saurashtra Coasts," 1956 (under publication).

23. Ketchum, B. H., Corwin, N. and Keen, D. J. — *Deep Sea Res.*, 1955, **2**, 172–81.
24. Marshall, S. M. and Orr, A. P. — *J. Mar. Biol. Assn. U.K.*, 1948, **27** (2), 360–79.
25. Miller, S. M. .. — *Bull. Marine Sci. Gulf and Caribbean*, 1952, **1** (4), 257–65.
26. Mortimer, C. H. .. — *J. Ecol.*, 1941, **29**, 280–329.
27. ———— .. — *Ibid.*, 1942, **30**, 147–201.
28. Orr, A. P. .. — *Great Barrier Reef Expedition Reports*, 1928–29, **2** (1).
29. Panikkar, N. K. and Jayaraman, R. — *Abstr. 8th Pacific Sci. Cong., Philippines*, 1953, 106–07; *Pacific Sci. Congr. Assn.* (Full paper in Press).
30. Phifer, L. D. and Thompson, T. G. — *J. Mar. Res.*, 1937, **1**, 34–53.
31. Prasad, R. R. .. — *Indian J. Fish.*, 1954, **1**, 1–36.
32. ———— .. — *Ibid.*, 1956, **3**, 1–42.
33. Rakestraw, N. W. and Smith, H. P. — *Bull. Bingh. Oceanogr. Colln.*, 1937, **6**, Art. 1.
34. Ramamurthi, S. .. — *J. Madras Univ.*, 1953, **23 B** (2), 148–63.
35. Redfield, A. C. .. — *Suppl. to Deep Sea Res., London*, 1955, **3**, 1–498.
36. ——, Smith, H. P. and Ketchum, B. — *Biol. Bull., Woods Hole*, 1937, **73**, 421–43.
37. Renn, C. E. .. — *Ibid.*, 1937, **72**, 190.
38. Riley, G. A. .. — *J. Mar. Res.*, 1937, **1**, 60–73.
39. Rittenberg, S. C., Emery, K. C. and Orr, W. L. — *Deep Sea Res.*, 1955, **3**, 23–45.
40. Rochford, D. J. .. — *I.P.F.C. Proc., Sec. II*, 1951 *a*, 1–8.
41. ———— .. — *Austr. J. Mar. Fresh. Res.*, 1951 *b*, **2** (1), 1–116.
42. Seiwell, H. R. .. — *Papers in Physical Oceanogr. and Meteorol.*, 1935, **3** (4), 1–56.
43. —— and Seiwell, G. E. .. — *Amer. Phil. Soc. Proc.*, 1938, **78**, 465–81.
44. Seshappa, G. .. — "Phosphate content of mud banks along Malabar Coast," *Nature, Lond.*, 1953 *a*, **171**, 526.
45. ———— .. — *Proc. Nat. Inst. Sci., India*, 1953 *b*, **19** (2), 257–79.
46. —— and Jayaraman, R. — *Proc. Ind. Acad. Sci.*, 1956, **43 B**, 288–301.
47. Stephenson, W. .. — *J. Mar. Biol. Assn. U.K.*, 1949, **28**, 371–80.
48. Sverdrup, H. U., Johnson, M. W. and Fleming, R. H. — *The Oceans, Their Physics, Chemistry and Biology*, 1942, 1087 pp., New York.
49. Tham Ah Kow .. — *Fishery Publ.*, 1953, **1** (4), London.
50. Various Authors .. — Parts I to IV. *Spl. Sci. Rep. Fisheries*, Nos. 54, 131 and 135, U.S. Fish. and Wild Life Service, 1954.
51. Waksman, S. A. and Renn, C. E. — "Decomposition of organic matter in Sea-Water. II. Factors influencing rate of decomposition," *Biol. Bull. Woods Hole*, 1936, **70**, 472–83.
52. ——, Hatchkiss, M., Carey, C. L. and Hardman, Y. — *J. Bact.*, 1938, **35**, 477–86.

A NEW REPORT OF A RUST [*PUCCINIA KUEHNII* (KRUEG) BUTLER] ON *ERIANTHUS MUNJA**

By S. L. Sharma, M.Sc.
(*Sugarcane Research Institute, Pusa, Bihar*)

Received April 13, 1957
(Communicated by Shri K. L. Khanna, F.A.Sc.)

I. Introduction

Puccinia kuehnii has been known to occur on *Saccharum spontaneum* since long (Butler, 1918). *Saccharum officinarum*, and four more plants, namely *Erianthus arundinaceus*, *Erianthus sara*, *Narenga porphyrocoma*, *Sclerostachya fusca*, formerly placed under this genus were found infected with this rust at one time or the other (Patel *et al.*, 1950; Chona and Munjal, 1950). So far, no rust has been reported to occur on *Erianthus munja* (Butler and Bisby, 1931; and Mundukar, 1938). This is, therefore, the first report of occurrence of a rust noted on this plant toward the end of September 1950.

II. Observations and Results

(*a*) *Occurrence.*—Observations on the occurrence of this rust made on 10 forms of *E. munja* and 7 of *E. arundinaceus* in the ensuing winter, showed that its various forms like those of *E. arundinaceus* behaved differentially towards this rust (Table I). Thus a clump of *E. munja* collected from high land was much more heavily rusted than the other collected from wet land although both of them were growing within 3 feet of each other near Cage House. The intensity of its infection on all forms of both the hosts, however, decreased with advent of summer.

(*b*) *Description.*—The rust on *E. munja* resembled very closely *Puccinia kuehnii* (Krueg) Butler, found on *E. arundinaceus* all the year round for last ten years (Padmanabhan and Rafay, 1942) and tallied with the general description given by Chona and Munjal (1950) except that pustules on both the hosts were from a tiny dot up to 5 mm. in length with a maximum width of

---

* 1. An abstract was published in *Proc. 39th Ind. Sci. Cong.*, 1952, Part III, 308–09.

2. This rust on *E. munja* has not been listed as yet in Ramakrishnan, K. and Subramanian, C. V., "The Fungi of India—A Second Supplement," *J. Madras Univ.*, 1952, **22B**, 1–65 and 163–82, and Subramanian, C. V. & Ramakrishnan, K., "List of Fungi 1952–56," *Ibid.*, 1956, 327–421, though it has been reported on several sugarcane varieties from Andhra and Uttar Pradesh. At Pusa (Bihar) this rust could not infect five sugarcane varieties [Sharma, S. L., "Pathogenicity of *Puccinia kuehnii* (Krueg) Butler on sugarcane varieties in Bihar," *Curr. Sci.*, 1952, **21**, 288].

TABLE I. *Incidence of rust* [*Puccinia kuehnii* (*Krueg.*) (*Butler*)] *on* Erianthus arundinaceus *and* E. munja

TABLE I. *Incidence of rust* [Puccinia kuehnii (*Krueg.*) (*Butler*)] *on* Erianthus arundinaceus *and* E. munja *at Pusa during* 1950–51

Sl. No. of Wild *Saccharum*	Name	Site	8-11-50	8-12-50	3-1-51	2-2-51	1-3-51	15-3-51	1-4-51	15-4-51	Remarks
	E. munja (High land)	Near the cage-house	3	3	3	3	2	2	1	1	Although both are growing within a yard of each other for last four years they are maintaining their characteristic hue of green colour. The high land Munja is bluish green while that from wet land is deep green.
	do. (Wet land)	do.	1	1	1	1	1	1	0	0	
	E. arundinaceus	do.	0	0	0	0	0	0	0	0	
	E. munja	Entomology-plot	..	..	5	5	4	3	3	3	In the shade of *Dalbergia sisso*.
W.S. 1	*E. arundinaceus*	Nursery	3	3	3	3	3	2	2	2	
W.S. 2	do.	do.	2	2	2	2	2	1	1	1	
W.S. 3	do.	do.	0	0	0	0	0	0	0	0	
W.S. 4	do.	do.	0	0	0	0	0	0	0	0	
W.S. 5	do.	do.	1	1	1	1	1	1	1	0	
W.S. 6	*E. munja*	do.	2	2	1	2	1	1	0	0	
W.S. 7	do.	do.	1	1	1	1	1	1	0	0	
W.S. 8	do.	do.	1	1	1	1	1	1	0	0	
W.S. 9	do.	do.	1	1	1	1	1	1	0	0	
W.S. 10	do.	do.	0	0	1	1	1	1	0	0	
W.S. 11	do.	do.	0	0	1	1	1	1	0	0	
W.S. 12	do.	do.	0	1	1	1	0	1	0	0	

Intensity of infection.—0 = Absent. 1 = Very light. 2 = Light. 3 = Moderate. 4 = Heavy. 5 = Very heavy.

TABLE II

Average size of 300 *uredospores of the rust on* E. munja *and those of* Puccinia kuehnii *found on* E. arundinaceus

Particulars	*E. munja*	*E. arundinaceus*
1. Length:		
(*a*) Range ..	52·6 μ–22·2 μ	45·9 μ–26·3 μ
(*b*) Average ..	35·0 μ	35·5 μ
(*c*) Standard error	0·31 μ	0·45 μ
2. Breadth:		
(*a*) Range ..	31·7 μ–18·9 μ	29·8 μ–20·8 μ
(*b*) Average ..	22·3 μ	24·1 μ
(*c*) Standard error	0·19 μ	0·08 μ

Difference between the length of uredospores from two hosts was not significant when tested by the formula $\frac{m_1 - m_2}{\sqrt{S_1^2 + S_2^2}}$ where m_1 and m_2 are the two means and S_1 and S_2 are their standard errors. It was however highly significant (at 1% level) in the case of breadth.

1 mm. and that they were not surrounded by a purple margin which later on turned dark coloured. On the contrary, very often pustules (Plate XIII, Fig. 1) were found in green islands on leaves yellowing due to age. Its teleuto stage has not been found as yet.

(*c*) *Size of uredospores.*—The average length calculated after measuring 300 uredospores taken from both the hosts was not found to be significantly different from each other though in the case of their breadth, the difference was significant at 1% level (Table II). But in view of the wide variation as regards the length and breadth of uredospores of this rust (Chona and Munja, 1950), much importance could not be attached to it. In both the cases, the spore-wall was nearly twice as thick at the top as at the sides.

(*d*) *Cross-inoculation.*—To further confirm that the two rusts were one and the same, cross-inoculations were done with the rust of *E. munja* (from entomological plot) on W.S.I. the most susceptible form of *E. arundinaceus* and *vice versa*. In absence of a spore-proof glass house in which all possibilities of an unwanted spore settling on the inoculated plants were eliminated, the chances of stray infection vitiating the result were minimised by marking the inoculated portion of a leaf at both the ends with India ink. If pustules appeared within the marks, then and then alone, they were taken as resulting

TABLE III

(*a*) *Details of cross-inoculations on* E. arundinaceus (*W.S.I.*) *and* E. munja (*from Entomology plot*) *with the rust from the other*

Date of inoculation	Rust from	Host inoculated	No. of leaves inoculated	No. of leaves infected	Date of first appearance of pustules	Nature of infection	Remarks
10-3-51	*E. munja*	*E. munja*	3	2	26-3-51	Heavy	..
do.	do.	*E. arundinaceus*	6	5	do.	do.	2-4-51; A few pustules on the sixth leaf just below the India ink mark.
do.	*E. arundinaceus*	do.	3	2	do.	do.	26-3-51; On the third leaf some pustules appeared below the India ink mark.
do.	do.	*E. munja*	6	4	do.	Light	..
13-3-51	*E. munja*	do.	2	0	..	..	2-4-51; One leaf developed pustules just below the mark.
do.	do.	*E. arundinaceus*	6	3	26-3-51	Light	2-4-51; (1) One of the infected leaves developed pustules below the mark also. (2) A few pustules appeared below the mark on another leaf. 20-4-51; One more leaf developed pustules below the mark.
do.	*E. arundinaceus*	do.	4	2	28-3-51	do.	..
do.	do.	*E. munja*	8	5	26-3-51	Heavy	..
17-3-51	*E. munja*	do.	2	0	..	..	29-3-51; One leaf dried. 2-4-51; The other also dried.
do.	do.	*E. arundinaceus*	9	4	31-3-51	Light	31-3-51; One pustule on another leaf below the mark. 2-4-51; One pustule each below the mark on two more leaves.
do.	*E. arundinaceus*	do.	3	2	2-4-51	do.	28-3-51; Two leaves dried. 7-4-51; Infected leaf developed pustules below the mark also. 22-4-51; Another leaf showed a few pustules below the mark.
do.	do.	*E. munja*	8	1	4-4-51	do.	..
11-4-51	*E. munja*	do.	4	3	28-4-51	do.	..
do.	do.	*E. arundinaceus*	11	9	24-4-51	do.	..
do.	*E. arundinaceus*	do.	3	3	27-4-51	do.	..
do.	do.	*E. munja*	10	3	28-4-51	do.	..

from the inoculum applied. That rust taken from one was able to infect the other (Table III *a*) though the percentage of inoculated leaves was irrespective of the source of inoculum, greater in the case of *E. arundinaceus* than in that of *E. munja* has been fairly well established by four sets of cross-inocu-

(*b*) *Summary of cross-inoculations of* Puccinia kuehnii (*Krueg*) *Butler on* E. arundinaceus *and* E. munja

Host	Source of inoculum	No. of leaves inoculated	No. of leaves infected	% of infected leaves
E. munja ..	*E. munja*	11	5	44·4
	E. arundinaceus	32	13	40·1
E. arundinaceus ..	*E. munja*	32	21	65·6
	E. arundinaceus	13	9	69·2

lations done in March and April 1951. Nearly 40–44% and 65–69% of leaves respectively of *E. munja* and *E. arundinaceus* developed rust pustules irrespective of the source of rust (Table III *b*).

III. CONCLUSION

It might, therefore, be stated that the rust on *Erianthus munja* was none other than *Puccinia kuehnii* found on *E. arundinaceus*. Its pathogenicity on the former, however, was not found to be as great as on the latter.

IV. ACKNOWLEDGEMENTS

The work was carried out at the Central Sugarcane Research Station, jointly financed by the Bihar Government and Indian Central Sugarcane Committee to whom grateful thanks are due. The author is also indebted to Shree K. L. Khanna, Director, Sugarcane Research and Development, Bihar, for placing the necessary facilities for this work at his disposal and to Shree M. M. Pandila for the help rendered to him in carrying out these investigations.

V. REFERENCES

1. Butler, E. J. .. *Fungi and Diseases in Plants*, 1918, 380–81 (Thacker Spink & Co., Calcutta).
2. ——— and Bisby, G. R. .. "Fungi of India," *Sci. Mono. No.* 1, *Imp. Coun. Agri. Res. India*, 1931, 232–33.
3. Chona, B. L. and Munjal, R. L. "*Puccinia kuehnii* (Krueg) Butler on Sugarcane," *Curr. Sci.*, 1950, **19**, 151–52.
4. Mundukar, B. B. .. "Fungi of India," *Supplement I, Sci. Mono. No.* 12, *Imp. Coun. Agri. Res. India*, 1938, 21–23.
5. Padmanabhan, S. Y. and Rafay, S. A. "Two new reports of fungi on *Saccharum officinarum* and *S. arundinaceum*," *Curr. Sci.*, 1942, **11**, 150–52.
6. Patel, M. K., Kamat, M. N. and Padhye, Y. A. "A new record of *Puccinia* on Sugarcane in Bombay," *Ibid.*, 1950, **19**, 120–22.

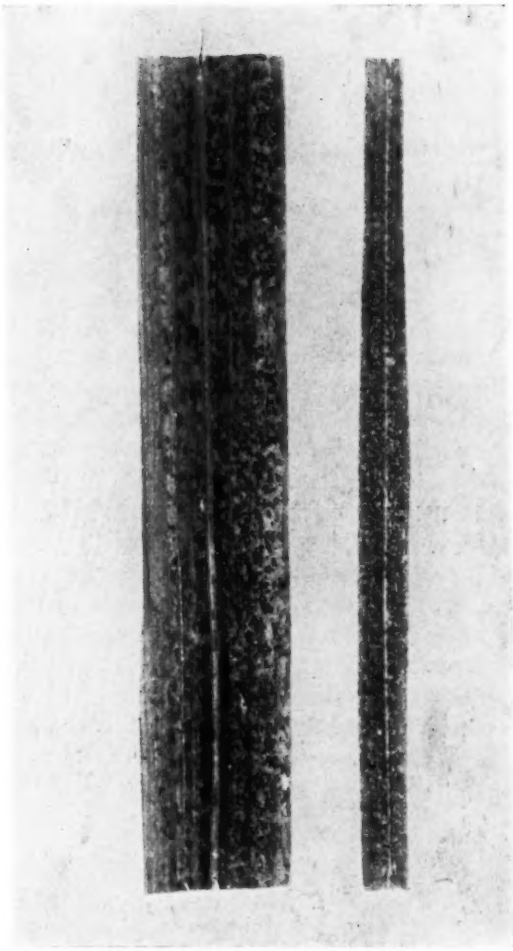


FIG. 1. Heavily rusted piece of *Erianthus arundinaceus* (*a*) and of *E. munja* (*b*) leaves.

INFLUENCE OF *FUSARIUM* CULTURE FILTRATES ON RESPIRATORY CHANGES IN COTTON

By M. Lakshmanan[1] and C. S. Venkata Ram[2]
(*University Botany Laboratory, Madras*)

Received May 10, 1957
(Communicated by Prof. T. S. Sadasivan, F.A.SC.)

Introduction

It is well known that parasitic fungi *in vivo* induce marked changes in the rate of respiration of the infected tissues (Allen, 1953). In the leaf of wheat infected with *Erysiphe graminis*, Allen and Goddard (1938) found a three-fold increase in respiration over the uninfected tissue. Sweet potato tissue infected with *Rhizopus tritici* gave a nine time increase in respiratory rate over the control (Weimer and Harter, 1921); respiration in sweet potato was also found to increase when treated to culture filtrates and mycelial extract of *Gibberella saubinetti* (Hellinga, 1940). In *Fusarium vasinfectum* infected cotton plants an increase in the respiratory rate over the non-infected tissue has been reported by Lakshmanan (1955). Many species of *Fusarium* commonly occurring in soil types are capable of infecting cotton plants to varying extent (Ray and McLaughlin, 1942; Venkata Ram, 1955) and it is also known that these Fusaria infest the vascular tracts of apparently healthy cotton plants (Rudolph and Harrison, 1945). It is, however, not clear how exactly these Fusaria affect host respiration and what changes are induced by the fungal metabolites in the normal physiological processes in the plant. As a preliminary enquiry into the problem, the respiratory changes in the plant tissue under the action of culture filtrates of twenty-one species of *Fusarium* were studied and the results are reported here.

Materials and Methods

Species of *Fusarium* were obtained from the Centraalbureau voor Schimmelcultures, Baarn, Holland. Fusaria were grown in 50 ml. Richard's medium in 250 ml. conical flasks at 27-29° C. and after three weeks' incubation the fungal mats were filtered and the filtrate tested for their action on tissue respiration of cotton. Stem sections (3 mm. thickness) obtained from 3-week old plants (susceptible variety Karunganni 2) were used for studying respira-

[1] Senior Fellow, Government of India.
[2] National Research Fellow, Ministry of Education, Government of India.

tion in the usual manner (Dixon, 1951; Lakshmanan, 1955). The strength of the culture filtrate was maintained at 50 per cent. concentration in Sorensen's phosphate buffer (pH 7·2). Respiratory rate of the tissue was measured in Barcroft's manometers every half hour over a period of three hours and the results were expressed as μl. O_2 uptake per hour per mg. dry weight of the tissue (Text-Fig. 1); the percentage increase in respiration over the control in species grouped under the different taxonomic divisions of the genus (Wollenweber and Reinking, 1935) is given in Table I.

DISCUSSION OF RESULTS

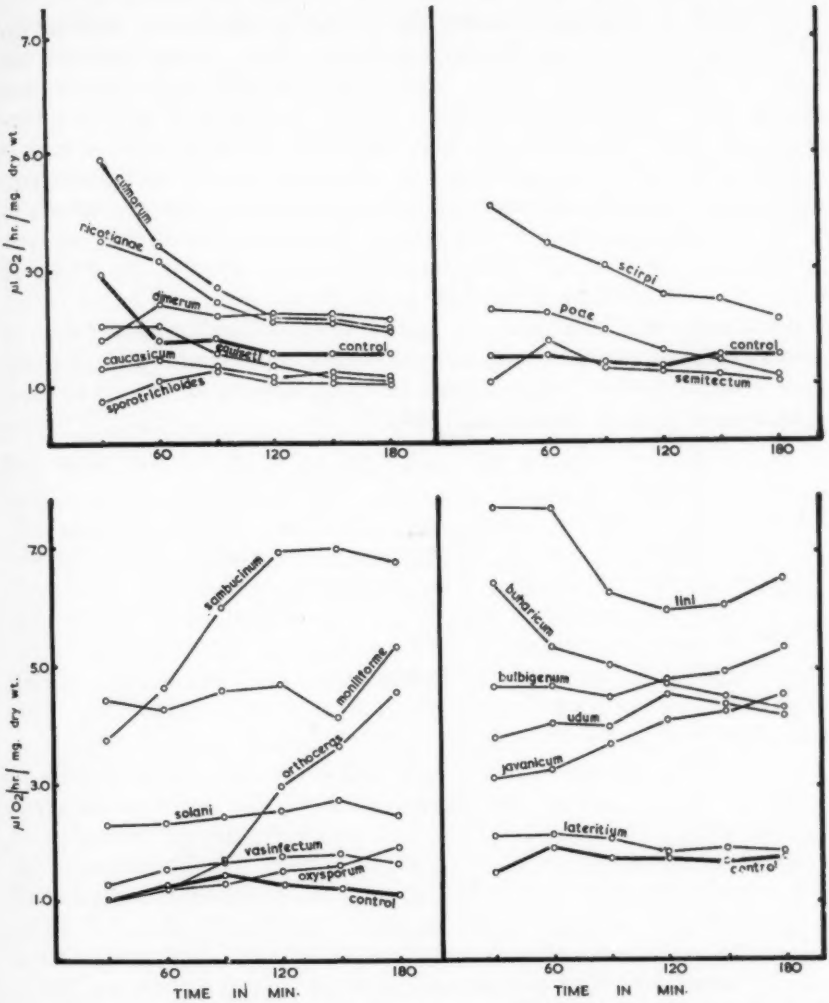
Culture filtrates of many Fusaria increased tissue respiration very markedly, whereas some did not bring about any appreciable change and a few others decreased the respiratory rate (Text-Fig. 1). Filtrates of *F. lini*, *F. sambucinum* and *F. moniliforme* elicited a high increase in respiration within the first half hour and the overall increase over the control was 300 per cent. (Table I). A 100 per cent. increase was observed in the respiratory rate of tissue under the action of culture filtrates of *F. bulbigenum* var. *lycopersici*; this fungus has already been reported as causing increase in CO_2 production in infected tissues of tomato (Gäumann, 1951). Only a slight increase was observed in the overall respiration of tissues treated to filtrates of the three pathogenic species on cotton, *F. vasinfectum*, *F. oxysporum* and *F. culmorum*. An inhibition in the respiratory rate of tissue was produced by the filtrates of *F. caucasicum*, *F. equiseti*, *F. semitectum* and *F. sporotrichioides* (Text-Fig. 1).

Broadly speaking, five types of respiratory changes in the tissue influenced by the culture filtrates of the Fusaria were distinguished.

(1) In tissues treated to filtrates of *F. dimerum*, *F. moniliforme*, *F. solani* and *F. udum* most of the increase in respiration occurred during the first 30–60 minute period but thereafter the respiratory rate remained steady (Text-Fig. 1).

(2) Filtrates of *F. buharicum*, *F. culmorum*, *F. lini*, *F. oxysporum* var. *nicotianae*, *F. poae* and *F. scirpi* elicited a high increase in respiration during the first hour but in this case the respiratory rate decreased sharply thereafter.

(3) Tissue respiration in filtrates of *F. bulbigenum* var. *lycopersici*, *F. javanicum*, *F. orthoceras* and *F. sambucinum* increased continuously with time and reached a maximum at the end of 150-80 minute period.



TEXT-FIG. 1. Showing the effect of culture filtrates of different species of Fusaria on the respiratory rates of healthy cotton tissues.

(4) Filtrates of *F. lateritium*, *F. oxysporum* and *F. vasinfectum* gave only slight increase in respiration and the rate of O_2 uptake by the tissue in these filtrates was comparable to that in the control (Text-Fig. 1).

(5) Inhibition of tissue respiration to varying degrees was observed in the case of filtrates of *F. caucasicum*, *F. equiseti*, *F. semitectum* and *F. sporotrichioides*.

Changes in substrate concentration, either qualitative or quantitative, are likely to influence tissue respiration (Allen, 1953). In the *Fusarium* culture filtrates some of the sugars, organic acids, amino acids, vitamins and growth factors present (Venkata Ram, 1956 *a*) were similarly tested for their action on tissue respiration of cotton, and these probably played a part in increasing the respiratory rate (Table I). However, none of these substances incited more than 100 per cent. increase in respiration, whereas filtrates of *F. lini*, *F. moniliforme* and *F. sambucinum* gave approximately 300 per cent. increase over the control. Other factors occurring either in the filtrate or certain reaction products of the host to the filtrate seemed to be concerned in stimulating respiration and only further work will indicate the nature of these substances. Sweet potato infected with *Ceratostomella fimbriata* produced reaction products which elicited very high increase in the rate of respiration in host tissue (Uritani *et al.*, 1954).

Production of toxins in the culture filtrates of the two vascular wilt Fusaria, *F. vasinfectum* and *F. oxysporum*, does not seem to be concerned with its low potentiality to increase respiration because even at greater dilutions these filtrates did not give greater increase in respiratory rate and also a very high increase in respiration was elicited by filtrates of *F. moniliforme* and *F. orthoceras*, known to contain high concentrations of fusaric acid, one of the wilt toxins (Yabuta, Kambe and Hayashi, 1935; Venkata Ram, 1956 *b*), which is known to inhibit respiration of cotton tissue (Lakshmanan, 1956). Many of the species not known to produce any toxin in culture inhibited respiration, as for example, *F. caucasicum*; other species which do not infect cotton but are pathogenic to other plants incited marked increase in respiratory rate, *viz.*, *F. lini*, and *F. udum* (Text-Fig. 1). It seems indicative, therefore, that the vascular wilt Fusaria in which physiologic specialization has progressed considerably, do not induce sudden or drastic changes in the normal host metabolism which is in itself essential for the continued existence and survival of the pathogen within the host. Respiratory rate of *F. vasinfectum* infected tissue in cotton was not found to increase over the uninfected tissue in 14-day old plants but increased after 21 days (Lakshmanan, 1955), which showed that no sudden change in host respiration took place immediately after the parasite had invaded the plant. This may explain why non-specific fungal pathogens, including Fusaria, causing root rots are able to kill the host much earlier than the highly specialized vascular wilt Fusaria. The increase in respiratory rate of tissues treated to filtrate of *F. vasinfectum* compared favourably with the increase in observed in 3-week old infected tissue of cotton (Lakshmanan, *loc. cit.*).

TABLE I

Percentage increase/decrease in respiration over the control of cotton tissue treated to culture filtrates of Fusaria, vitamins, organic and amino acids and growth substances

Fusarium spp.	Section	Concentration	Increase	Decrease
F. equiseti ..	Gibbosum	50%		18·88
F. scirpi ..	,,	,,	101·90	
F. sambucinum ..	Discolor	,,	364·10	
F. culmorum ..	,,	,,	52·14	
F. buharicum ..	,,	,,	189·40	
F. orthoceras ..	Elegans	,,	102·00	
F. oxysporum ..	,,	,,	16·01	
F. vasinfectum ..	,,	,,	30·51	
F. lini ..	,,	,,	309·50	
F. bulbigenum var. *lycopersici*	,,	,,	177·70	
F. udum ..	,,	,,	140·50	
F. oxysporum var. *nicotianae*	,,	,,	37·39	
F. solani ..	Martiella	,,	98·88	
F. javanicum ..	,,	,,	120·90	
F. caucasicum ..	,,	,,		31·52
F. poae ..	Sporotrichiella	,,	23·51	
F. sporotrichioides ..	,,	,,		42·23
F. moniliforme ..	Liseola	,,	288·80	
F. lateritium ..	Lateritium	,,	13·29	
F. dimerum ..	Eupinnotes	,,	14·25	
F. semitectum ..	Arthrosporiella	,,		11·28
Succinic acid		10^{-3} M	28·10	
Fumaric acid		,,	20·00	
Malic acid		,,	34·32	
Pyruvic acid		,,	57·85	
α-ketoglutaric acid		,,	53·29	
Indole acetic acid		10^{-4} M	138·00	
,,		10^{-5} M	116·25	
Phenylalanine		50 p.p.m.	35·23	
Tyrosine		,,	38·10	
Biotin		·3 p.p.m.	20·25	
,,		1 p.p.m.	30·15	
Thiamine		1 p.p.m.	10·10	
,,		10 p.p.m.	20·25	
Riboflavin		10 p.p.m.	10·89	
,,		50 p.p.m.	15·50	
Pyridoxine		10 p.p.m.	12·32	
,,		50 p.p.m.	16·50	

It might be suggested that the culture filtrates of pathogenic Fusaria are likely to contain not only specific toxic principles reproducing some or other of the typical wilt syndrome in the susceptible host but also growth factors playing an active role in the respiratory changes caused in host tissues. During active pathogenesis in living plants the liberation of such free auxins or the formation of specific intermediaries inhibiting the respiratory activity of the host tissue could be present to different degrees depending on the phase of infection.

The activity of the filtrates in influencing respiration differed markedly even between species belonging to the same taxonomic group (Table I), thus manifesting considerable diversity in species behaviour in Fusaria.

SUMMARY

Culture filtrates of twenty-one species of *Fusarium* were tested for their action on cotton tissue respiration. Filtrates of *F. buharicum*, *F. bulbigenum* var. *lycopersici*, *F. culmorum*, *F. dimerum*, *F. javanicum*, *F. lateritium*, *F. lini*, *F. moniliforme*, *F. orthoceras*, *F. oxysporum* var. *nicotianae*, *F. sambucinum*, *F. solani*, and *F. udum* elicited high increases in tissue respiration over the control, whereas that of *F. caucasicum*, *F. equiseti*, *F. semitectum* and *F. sporotrichioides* inhibited the respiratory rate. *F. lateritium*, *F. oxysporum* and *F. vasinfectum* caused only slight stimulation in tissue respiration.

Various organic acids, growth substances, amino acids and vitamins present in the culture filtrate when tested individually were found to increase respiratory rate and these probably played a part in stimulating respiration in *Fusarium* infected tissue.

ACKNOWLEDGEMENTS

We thank Prof. T. S. Sadasivan, D.Sc. (LOND.), F.N.I., for his help and encouragement during the course of this investigation. We are thankful to the Government of India for the award of Fellowships.

REFERENCES

Allen, P. J. .. "Toxins and tissue respiration," *Phytopathology*, 1953, **43**, 221–29.

——— and Goddard, D. R. .. "A respiratory study of powdery mildew of wheat," *Amer. J. Bot.*, 1938, **25**, 613–21.

Dixon, M. .. "*Manometric Methods*," Cambridge University Press, 1951.

Gäumann, E. .. "Some problems of pathological wilting in plants," *Adv. Enzymol.*, 1951, **11**, 401–37.

Hellinga, J. J. A. .. "On the effect of substances produced by fungi on the respiration of the tissue of potato tubers, II," *Proc. Kon. Ned. Acad. Wetenschap.*, 1940, **43**, 267–76.

Lakshmanan, M. .. "Respiratory studies on vascular wilts," *Proc. Ind. Acad. Sci.*, 1955, **41 B**, 151-54.

——— .. "Host parasite relationship in plant wilts with special reference to respiration," *Doctoral Thesis*, Madras University, 1956.

Ray, W. W. and McLaughlin, J. H. "Isolation and infection tests with seed and soil-borne cotton pathogens," *Phytopathology*, 1942, **32**, 233–38.

Rudolph, B. A. and Harrison, G. J. "The invasion of the internal structures of cotton seed by certain Fusaria," *Ibid.*, 1945, **35**, 542–48.

Uritani, I., Akazawa, T. and Uritani, M. "Increase of respiratory rate in sweet potato tissue infected with black-rot," *Nature, Lond.*, 1954, **174**, 1060.

Venkata Ram, C. S. .. "Variation and variability in Fusaria with reference to taxonomy," *Proc. Ind. Acad. Sci.*, 1955, **41 B**, 123–31.

——— .. "Detection of growth factors in culture filtrates of Fusaria," *Curr. Sci.*, 1956 *a*, **25**, 329–30.

——— .. "Production of fusaric acid by *Fusarium orthoceras in vitro*," *Experientia*, 1956 *b* (In press).

Weimer, J. L. and Harter, L. L. .. "Respiration and carbohydrate changes produced in sweet potatoes by *Rhizopus tritici*," *J. agric. Res.*, 1931, **21**, 627–35.

Yabuta, T., Kambe, K. and Hayashi, T. "Biochemistry of the bakanae fungus, I. Fusaric acid, a new product of the bakanae fungus," *J. agric. Chem. Soc., Japan*, 1934, **10**, 1049–69.

Wollenweber, H. W. and Reinking, O. A. "*Die Fusarien, ihre Beschreibung Schadwirkung und Bekampfung*, Paul Parey, Berlin, 1935.

THE METABOLISM OF SERINE IN PYRIDOXINE DEFICIENCY

By G. B. Nadkarni and A. Sreenivasan, F.A.Sc.

(*Department of Chemical Technology, University of Bombay*)

Received May 10, 1957

Formation of ethanolamine from serine and glycine has been shown in the animal organism (Stetten, 1942; Greenberg and Harris, 1950; Weissbach *et al.*, 1950; Jonsson and Mosher, 1950; Levine and Tarver, 1950). Evidence is also available to show that ethanolamine is an intermediate in the biosynthesis of choline (Arnstein, 1951; Sprinson and Coulon, 1954; Elwyn *et al.*, 1955). Amines are known to be formed as a result of decarboxylation of amino acids (Gale, 1946, Blaschko, 1945). The presence of ethanolamine in the urine of rats (Stein, 1953) and the increase in its excretion with administration of serine (Fishman and Artom, 1945; Luck and Wilcox, 1953) would indicate that serine is the source of ethanolamine. Absence of label in the hydroxymethyl group of ethanolamine isolated after feeding of carboxyl labelled glycine would also suggest that glycine has to be transformed to serine prior to decarboxylation (Greenberg and Harris, 1950). Nord (1919) had shown that an aerobic bacteria produced ethanolamine from serine in putrefying meat. Ethanolamine formation is also reported in intestinal micro-organisms (Melnykowicz and Johansson, 1955).

Although pyridoxine, as pyridoxal phosphate is known to be the coenzyme of amino acid decarboxylases in bacteria (Snell, 1953; Mandeles *et al.*, 1954), its participation in serine decarboxylation in the animal organism is not established. However, from an observed decrease in choline biosynthesis in the vitamin B_6-deficient rat, Stekol *et al.* (1952, 1953) inferred a function for vitamin B_6 in serine decarboxylation.

Serine deamination in *Neurospora* is known to be influenced by pyridoxine (Yanofsky and Reissig, 1953); again, this effect has not been demonstrated in the animal organism. Glycine to serine conversion requires participation of pyridoxal phosphate and has been extensively studied (Deodhar and Sakami, 1952; Blakley, 1956; Alexander and Greenberg, 1955).

The present work relates to a study of serine metabolism in vitamin B_6 deficiency in rats. It is shown that there is no apparent effect of this vitamin on serine decarboxylation *in vitro*, while serine deamination, as well as glycine to serine conversion are influenced by vitamin B_6.

Experimental

Preparation of Tissue

The rat was killed by decapitation and the liver was quickly removed and chilled. It was homogenised to a 20 per cent. suspension in ice-cold distilled water using a Potter-Elvehjem all-glass homogeniser.

Manometric Assay of Serine Decarboxylation

Assays were carried out in Warburg flasks in a nitrogen atmosphere. In preliminary experiments, optimum conditions with respect to pH and substrate concentration, were ascertained. The procedure finally adopted was as follows: The main compartment of the Warburg flask contained 1·0 ml. each of 0·1 M phosphate buffer, pH 6·0 and of the liver homogenate. The side-arm contained 0·5 ml. of 0·1 M DL-serine solution or distilled water in the case of controls. The contents of the side-arm were tipped in after initial equilibration period of 10 minutes at 37 ° C., during which nitrogen gas was passed through the flask. The centre cup contained 0·5 ml. of 1 N H_2SO_4 which was added to the main chamber to stop the reaction. The acid was added when there was no further change in manometric levels, which usually occurred within about 40 minutes.

In some experiments filter-paper strips soaked in 10 per cent. KOH were inserted into the centre cup instead of the 1 N H_2SO_4 and it was always observed that in these cases there was no significant change in pressure, indicating absorption of the carbon dioxide liberated.

Correction for CO_2 retained in the liquid phase was negligible at the prevailing acidic pH.

The ethanolamine formed as a result of serine decarboxylation was identified on circular paper chromatograms in the following manner: The reaction mixture in the Warburg flask was taken in a test-tube and heated on a boiling water-bath for 10 minutes. It was then centrifuged and the supernatant used for chromatography. The technique was similar to that of Giri and Rao (1952). Samples were spotted side by side, with solutions of serine and ethanolamine. A somewhat diffuse band characteristic of ethanolamine was observable. The R_f values for ethanolamine were 0·60 and 0·75 in phenol-water (80 : 20) and butanol-acetic acid-water (40 : 10 : 50) as solvents respectively. The corresponding R_f values for serine were 0·40 and 0·50 respectively.

Serine Deamination

1·0 ml. of the liver homogenate was incubated with 1·0 ml. of 0·1 M phosphate buffer pH 7·4 and 1·0 ml. of 0·05 M serine solution at 37° C.

for an hour. At the end of the incubation period, the reaction was stopped and the mixture was deproteinised by adding 1·0 ml. of 20 per cent. trichloroacetic acid solution. An aliquot of the supernatant after centrifugation was used for determinations of ammonia and pyruvic acid formed. A control experiment without serine was performed side by side. *Ammonia* was determined by direct Nesslerization (Johnson, 1941) and *Puyrvic acid* by the method of Friedemann and Haughen (1943).

Glycine to Serine Conversion

1·0 ml. of the liver homogenate was incubated under toluene with 0·1 M phosphate buffer pH 7·0, 0·5 ml. of 0·2 M glycine, 0·5 ml. of 0·2 M sodium formate, 0·4 ml. of 0·05 M sodium citrate and 0·1 ml. of 0·05 M sodium fumarate at 37° C. for 5 hours. In a number of preliminary experiments it was noted that this system was the best for following serine synthesis from glycine. The citrate-fumarate mixture served as a sparking system (Fatterpaker, Marfatia and Sreenivasan, 1951). A control experiment with 0·5 ml. of distilled water substituting the glycine solution was run alongside. At the end of the incubation period 0·5 ml. of 1 N H_2SO_4 was added and the mixture was deproteinised by heating on a boiling water-bath for 5 minutes. An aliquot of the supernatant after centrifugation was used for the estimation of serine. The difference in serine values with and without added glycine represented net synthesis of serine.

Serine was assayed microbiologically with *Leuconostoc mesenteroides* P-60 (Steele *et al.*, 1949) and with *Lactobacillus casei* (Alexander, Beckner and Elvehjem, 1953). The assays were conducted in a final volume of 2·0 ml. per tube. The standard solution of *dl*-serine contained 20 μg. per ml. and the standard curve was obtained between 0–20 μg. of serine for every assay. The results are expressed as *l*-serine with appropriate reference to the standard curve. The latter organism was found to be more suitable and was therefore employed generally.

Assay of Pyridoxine

1·0 g. of homogenised liver was hydrolysed in 1 N NaOH by autoclaving for an hour at 15 lb. pressure, in a volume of 10 ml. The alkaline extract was cooled, neutralised with hydrochloric acid and made up to 50 ml. volume. Aliquots were used for the assay of pyridoxine with *Neurospora sitcphila* (Stokes *et al.*, 1943). The range of the assay was 0·1–1·0 μg.

Total Liver Nitrogen

1·0 ml. of the liver homogenate was taken in a Kjeldahl flask to which was added 1·0 g. of a salt mixture containing potassium sulphate and copper

sulphate (10 : 1) and 2·0 ml. of sulphuric acid (A.R.). The digestion was carried out over a micro-burner for 5–6 hours till a clear solution was obtained. The solution was diluted with water, neutralised slowly in the cold with NaOH and made up to volume. Colour was then developed by direct Nesslerization. To 1·0 ml. of the aliquot was added 1·0 ml. water, 2·0 ml. 2 N NaOH and 1·0 ml. of Nessler's reagent. Colour measurements were made on a Klett-Summerson photoelectric colorimeter with 420 mμ. filter in position. A standard curve was obtained with a solution of NH_4Cl in the range 0–15 μg. of nitrogen for reading off the values for total nitrogen.

Liver Choline

Free Choline.—2–3 g. of homogenised liver in 50 ml. of distilled water were heated at 100° C. for one hour (Leucke and Pearson, 1944). The mixture was filtered and aliquot used for determination of free choline.

Total Choline.—A suspension of the homogenised liver was hydrolised with 3 per cent. H_2SO_4 by autoclaving it for 2 hours at 15 lb. pressure (Horowitz and Beadle, 1943). It was cooled, neutralised with barium carbonate and made to volume. It was then filtered and the filtrate used for determination of total choline.

5–10 ml. aliquots prepared as above were passed through previously activated DeCalso columns which were then washed with distilled water. Choline adsorbed was next eluted with 10 ml. of 5 per cent. NaCl solution.

An aliquot of the eluate containing about 10–20 μg. of choline was used for precipitation as periodide by the method of Appleton *et al.* (1953). The periodide was centrifuged in specially tapered tubes and dissolved in redistilled ethylene dichloride. The extent of absorption was measured on a Beckman DU spectrophotometer at 365 mμ and results read off from a standard curve for choline in the range 0–15 μg.

Pyridoxine Deficiency in Rats

Young rats (Wistar) weighing 80–100 g. were fed on a purified diet of the following percentage composition (based on Meister *et al.* 1953; Beaton *et al.*, 1953); alcohol-extracted casein 18, starch 60, sucrose (vitaminised) 10, salt mixture (U.S.P. IV) 4, sesame oil 6, and shark liver oil 2. Vitamins included in the diet were (as mg./kg.): Thiamine HCl 6, riboflavin 10, niacin 30, calcium pantothenate 30, *p*-aminobenzoic acid 100, biotin 1, folic acid 5, vitamin K 3 and vitamin B_{12} 0·5.

One group of animals was reared on this vitamin B_6-deficient diet while a second group had the same diet with 10 mg./kg. pyridoxine HCl added.

To the deficient animals desoxypyridoxine was given intraperitoneally to hasten deficiency. This was at 0·5 mg. per rat on alternate days. Control rats were given mock injections of saline. There was no weight increase after the second week in the deficient group. Typical signs of deficiency such as dermatitis on the forepaws and the snout were noticed in 3–4 weeks' time. Animals were sacrificed for the various determinations after the 5th week. All results reported are averages from at least four different animals for each group.

RESULTS

Serine decarboxylation was rapid and most of the CO_2 was produced within the first 20–30 minutes of incubation and there was no further change in manometric levels after 40 minutes. The final concentration of the *dl*-serine added to the incubation mixture was 0·016 M (0·5 ml. of 0·1 M *dl*-serine in 3·0 ml.) and as may be seen from Table I, maximal activity was obtained at this concentration.

TABLE I

Effect of substrate concentration on serine decarboxylation

Final concentration of serine	Decarboxylation of serine QCO_2 (μl. CO_2 per mg. N/30 min.)
0·003 M	0·84
0·008 M	1·99
0·016 M	3·53
0·025 M	3·80
0·033 M	3·83

The optimal pH for reaction was found to be 6·0 (Table II). Buffers with pH ranging from 4·0–7·4 were used.

TABLE II

Effect of pH on serine decarboxylation

pH	Buffer	Decarboxylation of serine QCO_2 (μl. CO_2 per mg. N/30 min.)
4·0	0·2 M Acetate	2·42
5·0	,,	2·66
5·6	,,	3·35
6·0	0·1 M Phosphate	3·64
7·0	,,	1·97
7·4	,,	1·35

In view of the known activation of certain amino acid decarboxylases by pyridoxal phosphate (Gale, 1946; Roberts and Frankel, 1951; Mandeles *et al.*, 1954), its effect was studied on the present system. A pyridoxal phosphate preparation was obtained by a method similar to that of Umbreit and Waddel (1949) and Hurwitz (1953). 50 μg. of pyridoxal hydrochloride in 1·0 ml. of 0·1 M phosphate buffer pH 7·4, were incubated with 1·0 ml. of liver homogenate, 0·4 ml. of 0·05 M sodium citrate and 0·1 ml. of 0·05 M sodium fumarate, for three hours at 37° C. The mixture was deproteinised by heating on a boiling water-bath for 5 minutes, centrifuged and the supernatant used. Addition of this pyridoxal phosphate preparation at a concentration equivalent to 20 μg. of pyridoxal did not make any difference to serine decarboxylation but had a slight (9 per cent.) activating effect on synthesis of serine from glycine.

The inhibitory action, if any, of added hydroxylamine HCl on these enzyme systems was also studied.

TABLE III

Hydroxylamine inhibition studies

Addition to normal system	Serine decarboxylation QCO_2 (μl. CO_2 per mg. N/30 min.)	Glycine to serine synthesis (μ moles of serine per mg. N)	Serine deamination (μ moles of NH_3 per mg. N)
None	3·86	0·96	3·06
Hydroxylamine (10^{-3} M)	3·80	0·77	0·49

Hydroxylamine addition inhibited glycine to serine conversion by 20 per cent. and serine deamination by 85 per cent. However, it had no effect on serine decarboxylation. The insensitivity of serine decarboxylase to hydroxylamine could not be attributed to a pyridoxal phosphate coenzyme being firmly bound to the enzyme since the activity of this enzyme is not influenced in dietary vitamin B_6 deficiency (Table IV). On the other hand, there was marked reduction of activity in glycine to serine synthesis and in serine deamination as a result of vitamin B_6 deficiency.

Data on liver analyses of normal and pyridoxine-deficient animals are shown in Table V. Of significance are the values for free and total choline. Any effect on the decarboxylation of serine should be reflected in the values

TABLE IV

Effect of pyridoxine deficiency

Group	Serine decarboxylation QCO_2 (μl. CO_2 per mg. N/30 min.)	Serine deamination		Glycine to serine synthesis (μ moles of serine per mg. N)
		Pyruvic acid	Ammonia	
		μ moles per mg. N		
$-B_6$	3·87±0·14	3·08±0·04	3·03±0·05	0·63±0·02
$+B_6$	3·83±0·11	4·35±0·02	4·38±0·04	1·14±0·03

of choline. The values for total choline are reduced in the deficient rats but the levels of free choline seem to be almost unaltered.

DISCUSSION

Mammalian decarboxylases are of significance in the elaboration of specialised substances such as histamine, taurine, 5-hydroxytryptamine, ethanolamine, etc. The formation of ethanolamine provides the ethanolamine moiety of choline. The production of acetate from ethanolamine helps in the biosynthesis of long chain complex substances like sphingosine, fatty acids and cholesterol (Sprinson and Coulon, 1954). Ethanolamine also serves as an intermediate in the alternate serine to glycine pathway (Weissbach and Sprinson, 1953). Since serine-β-carbon can provide the methyl group of choline, serine becomes concerned with the biosynthesis of both the methyl and ethanolamine moieties of choline.

The present studies indicate that serine decarboxylase of rat liver is unique in that it is apparently not influenced by pyridoxine deficiency. The role of pyridoxal phosphate has been established in almost all bacterial amino acid decarboxylases and in some mammalian decarboxylases. The mammalian decarboxylases studied do not all behave similar to bacterial decarboxylases. Thus, while normally decarboxylases function at acidic pH values, 5-hydroxytryptophan decarboxylase shows an optimum pH of 8·1 (Clark *et al.*, 1954). Again, inhibition of glutamic acid decarboxylase (Roberts and Frankel, 1951) by hydroxylamine could be reversed by pyridoxal phosphate, but such a reversal could not be shown with 5-hydroxytryptophan decarboxylase (Clark *et al.*, 1954; Beiler and Martin, 1954). However, it has been shown recently that pyridoxal phosphate does have a function in 5-hydroxytryptophan decarboxylase (Buxton and Sinclair, 1956). Bacterial histidine decarboxylase (Rodwell, 1953) was also not considered as requiring pyridoxal phosphate,

TABLE V

Liver analyses

Group	Liver weight per 100 g. body weight	Pyridoxine in liver μg./g. fresh weight	Liver nitrogen		Liver choline	
			Per cent. fresh weight	Per cent. dry weight	Free mg./g. fresh weight	Total
$-B_6$	4·58±0·42	4·10±0·02	3·43±0·14	11·7±1·4	0·21±0·01	5·50±0·05
$+B_6$	3·37±0·40	6·91±0·01	3·91±0·24	13·6±0·9	0·23±0·01	5·83±0·03

but this requirement has since been shown (Guirard and Snell, 1954). Although the possibility exists that a function for pyridoxal phosphate in serine decarboxylation by the rat liver enzyme could not be demonstrated because of firmly bound coenzyme further work is needed to ascertain this possibility.

Serine when administered is excreted as ethanolamine in urine (Fishman and Artom, 1945; Luck and Wilcox, 1953). Similarly, administration of cysteine (Awapara and Wingo, 1953; Blaschko *et al.*, 1953) results in excretion of taurine. It is reported that in vitamin B_6 deficiency, the excretion of taurine is considerably reduced, though such an effect is not evident on ethanolamine excretion (Blaschko *et al.*, 1953).

The results on liver levels of choline (Table V) show a definite influence of vitamin B_6 on total choline content but not on the free choline to the same extent.

Although a function for vitamin B_6 in serine decarboxylation seems doubtful the present experiments have clearly brought out a relationship of this vitamin to glycine-serine conversion and in serine deamination. The participation of vitamin B_6 in glycine to serine transformation has been reported (Lascelles and Woods, 1950; Deodhar and Sakami, 1952; Blakley, 1955), and is explained on the basis of a Schiff base formation (Alexander and Greenberg, 1955). Involvement of vitamin B_6 in the deamination of serine has only been shown hitherto in micro-organisms.

SUMMARY

Deficiency of vitamin B_6 in rats had no effect on serine decarboxylation, though, serine deamination and glycine to serine synthesis were reduced.

REFERENCES

Alexander, J. C., Beckner, W. and Elvehjem, C. A. — *J. Nutr.*, 1953, **51**, 319.

Alexander, N. and Greenberg, D. M. — *J. Biol. Chem.*, 1955, **214**, 821.

Appleton, H. D., LaDu, B. N., Jr., Levy, B. B., Steele, J. M. and Brodie, B. B. — *Ibid.*, 1953, **205**, 803.

Arnstein, H. R. V. .. *Biochem. J.*, 1951, **48**, 27.

Awapara, J. and Wingo, W. J. .. *J. Biol. Chem.*, 1953, **203**, 189.

Beaton, J. R., Beare, J. L., White, J. M. and McHenry, E. W. — *Ibid.*, 1953, **200**, 715.

Beiler, J. M. and Martin, G. J. .. *Ibid.*, 1954, **211**, 39.

Blakley, R. L. .. *Biochem. J.*, 1955, **61**, 315.

Blaschko, H. .. *Adv. in Enzymol.*, 1945, **5**, 67.

Blaschko, H., Dutta, S. P. and Harris, H. — *Brit. J. Nutr.*, 1953, **7**, 364.

Buxton, J. and Sinclair, H. M. .. — *Biochem. J.*, 1956, **62**, 27 p.

Clark, C. T., Weissbach, H. and Udenfriend, J. — *J. Biol. Chem.*, 1954, **210**, 139.

Deodhar, S. and Sakami, W. .. — *Fed. Proc.*, 1952, **12**, 195.

Elwyn, D., Weissbach, S., Henry, S. and Sprinson, D. B. — *J. Biol. Chem.*, 1955, **213**, 281.

Fatterpaker, P., Marfatia, U. and Sreenivasan, A. — *Nature*, 1951, **167**, 1067.

Fishman, W. H. and Artom, C. .. — *Proc. Soc. Exptl. Biol. Med.*, 1945, **60**, 288.

Friedemann, T. E. and Haughen, G. E. — *J. Biol. Chem.*, 1943, **147**, 415.

Gale, E. F. .. — *Adv. in Enzymol.*, 1946, **6**, 1.

Giri, K. V. and Rao, N. A. N. .. — *Curr. Sci.*, 1952, **21**, 11.

Greenberg, D. M. and Harris, S. C. — *Proc. Soc. Exptl. Biol. Med.*, 1950, **75**, 683.

Guirard, B. M. and Snell, E. E. .. — *J. Am. Chem. Soc.*, 1954, **76**, 4745.

Horowitz, N. H. and Beadle, G. W. — *J. Biol. Chem.*, 1943, **150**, 325.

Hurwitz, J. .. — *Ibid.*, 1953, **205**, 935.

Johnson, M. J. .. — *Ibid.*, 1941, **137**, 575.

Jonsson, J. and Mosher, W. A. .. — *J. Am. Chem. Soc.*, 1950, **72**, 3316.

Lascelles, J. and Woods, D. D. .. — *Nature*, 1950, **166**, 649.

Leucke, R. W. and Pearson, P. B. — *J. Biol. Chem.*, 1944, **155**, 507.

Levine, M. and Tarver, M. .. — *Ibid.*, 1950, **184**, 427.

Luck, J. M. and Wilcox, A. .. — *Ibid.*, 1953, **205**, 859.

Mandeles, S., Koppleman, R. and Hanke, M. E. — *Ibid.*, 1954, **209**, 327.

Meister, A., Morris, H. P. and Tice, S. V. — *Proc. Soc. Exptl. Biol. Med.*, 1953, **82**, 301.

Melnykowicz, J. and Johansson, K. R. — *J. Exp. Med.*, 1955, **101**, 507.

Nord, F. F. .. — *Biochem. Z.*, 1919, **95**, 281.

Roberts, E. and Frankel, S. .. — *J. Biol. Chem.*, 1951, **188**, 789.

Rodwell, A. W. .. — *J. Gen. Microbiol.*, 1953, **8**, 233.

Snell, E. E. .. — *Physiol. Rev.*, 1953, **33**, 509.

Sprinson, D. B. and Coulon, A. .. — *J. Biol. Chem.*, 1954, **207**, 585.

Steele, B. F., Sauberlich, H. E., Reynolds, M. S. and Baumann, C. A. — *Ibid.*, 1949, **177**, 533.

Stein, W. H. .. — *Ibid.*, 1953, **201**, 45.

Stekol, J. A., Weiss, K. and Weiss, S. — *Arch. Biochem. Biophys.*, 1952, **36**, 5.

——, Weiss, S., Smith, P. and Weiss, K. — *J. Biol. Chem.*, 1953, **201**, 299.

Stetten, D., Jr. .. — *Ibid.*, 1942, **144**, 501.

Stokes, J. L., Larsen, A., Woodward, C. R., Jr. and Foster, J. W. — *J. Biol, Chem.*, 1943, **150**, 17.

Umbreit, W. W. and Waddel, J. G. — *Proc. Soc. Exptl. Biol. Med.*, 1949, **70**, 293.

Weissbach, S., Elwyn, D. and Sprinson, D. B. — *J. Am. Chem. Soc.*, 1950, **72**, 3316.

——— and Sprinson, D. B. .. *J. Biol. Chem.*, 1953, **203**, 1031.

Yanofsky, C. and Reissig, J. L. .. *Ibid.*, 1953, **202**, 567.

769–57. Printed at The Bangalore Press, Bangalore City, by C. Vasudeva Rao, Superintendent, and Published by the Indian Institute of Science, Bangalore-3, India.

NOTES ON SOME FUNGI FROM SOUTH INDIA—VI

By T. S. Ramakrishnan, F.A.Sc.

Received May 13, 1957

Oidium sp.

On leaves and shoots of *Punica granatum* L. (Lythraceæ), Coonoor, 30-3-1956, T. S. R.

An entire hedge of this host plant (flowering variety) was severely affected. An ashy powdery growth developed on the shoots and both surfaces of the leaves. Tender leaves were damaged to a greater extent. These were reduced in size and distorted. The conidia were formed in short chains and were hyaline, elliptical or barrel-shaped, measuring 20–26×9–14 μ. Swollen globoid haustoria were developed in the epidermal cells of the host. The conidial stage alone was present and perithecia were not observed.

Leveillula taurica (Lev.) Arn.

On leaves of *Rauwolffia serpentina* Benth. (Apocynaceæ), Velimalai (Kanyakumari District), 13–2–1957, T. S. R.

The host plant grows wild and is also planted in this locality. On a large number of the plants the mildew was evident on the lower surface of the leaves in restricted or extensive patches. The infected areas were yellowish in colour when viewed from the upper surface. The conidia had the characteristic club-shape and measured 48–65×11–17 μ.

Taphrina maculans Butl.

Butler, E. J., *Ann. Mycol.*, 1911, **9**, 36–39.

On leaves of *Curcuma longa* L. (Zingiberaceæ), Ayarkunnam (Kottayam), 15-11-1956, T. S. R.

The leaf-spot caused by this pathogen was severe in some of the plots. Each of the leaves was covered by numerous reddish brown or golden brown irregular blotches. The disease has not been recorded so far from any place south of Poona in the west or Samalkota in the east. The turmeric plots in the Cauvery Valley in the Coimbatore and Thiruchirapalli Districts are free from this disease. The occurrence of the disease on the west coast indicates that a moist climate favours the disease.

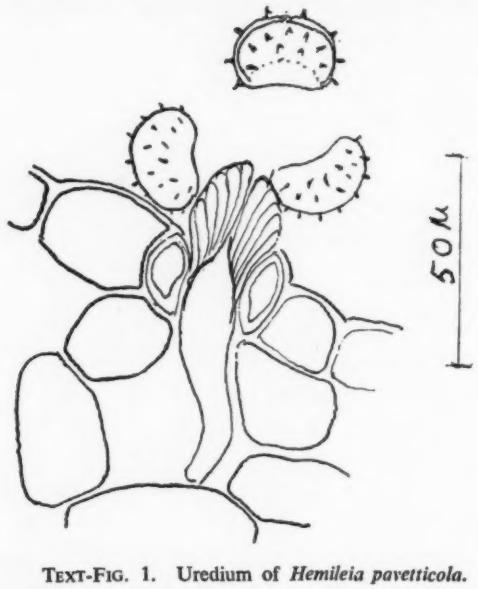
Hemileia pavetticola Maubl. and Roger

Bulletin Soc. Mycol. France, 1938, **54**, 48–54.

On living leaves of *Pavetta tomentosa* Roxb. (Rubiaceæ), Puthupalli (Kottayam), 10–10–1956, T. S. R.

This rust was common on the leaves of young plants. The uredosori formed orange-coloured powdery growths on the lower surface of the leaves sometimes covering the entire surface. The urediospores were reniform to elliptical with the inner side smooth and the rest prominently aculeate. A stout orange coloured hypha was present in the substomatal air-space. On emergence through the stoma this bore the characteristic swollen structures. From these sterigmata were produced bearing the spores.

The urediospores germinated within 12 hours forming long branched germ tubes. Telia were not present. The uredia were infected by a hyperparasite. The affected sori were whitish and overgrown by a *Fusarium* sp.



TEXT-FIG. 1. Uredium of *Hemileia pavetticola*.

Pellicularia filamentosa (Pat.) Rogers

Rogers, D. P., The genus *Pellicularia*, *Farlowia*, 1943, **1**, 95–118.

On leaves, stem and spikes of *Piper nigrum* L. (Piperaceæ), Kanjirapalli (Kerala), 22–10–1956, T. S. R.

This pathogen infects the leaves, stem and spikes of pepper. On the leaves two types of symptoms were evident. On some of the leaves the fungus formed a repeatedly branched filmy growth of mycelium on the lower

surface. The overgrown areas were blackened. Some leaves had become detached from the shoot but were hanging suspended by the mycelial strands. In the tissues of the blackened portions the hyphæ had penetrated and ramified in the mesophyll. In some vines the shoots and spikes also were overgrown by the fungus and were partially blackened. Other leaves were green with a whitish powdery growth on the lower surface. The powdery growth was made up of basidia and basidiospores.

The extent of damage, caused, varied in different vines. In one garden the vines were being grown on concrete posts. In some of these vines over 20 per cent. of the leaves at the base were blackened and rotting. Usually shade is considered to be favourable for the spread of disease caused by this fungus. In these instances shade did not come in. The contact with the soil, made by some of the shoots, might have led to the infection originating from the soil and spreading up.

The fungus was readily isolated from the leaves into pure culture. A rapid growth with loose aerial hyphæ and compact mat-like formation on the substratum formed on oat-agar in three days. A few clusters of chocolate brown sclerotia, 2–4 mm. in diameter, were formed.

On pepper leaves, infection was initiated in 48 hours after inoculation and some of the leaves rotted within a week. On some leaves the characteristic white powdery growth developed on the lower surface and such leaves remained green. On the spikes and shoots also similar growths occurred.

The pathogen readily passed on to rubber. On tender leaves water-soaked spots formed in 48–72 hours. The filmy growth developed on the lower surface of the inoculated leaves. These leaves became shrivelled and were shed. On older leaves the fungus formed a powdery pellicle on the lower surface and basidia and basidiospores were evident. Gradually the fungus spread over the shoots forming a thin white growth. The infected leaves separated from the shoots but were held together by the mycelial webs.

P. filamentosa was observed on the leaves of *Gardenia grandiflora* and *Elephantopus scaber* L. in Kanjirapalli. It is probable that more than one strain is prevalent in this locality as shown by their cultural characters.

Cercospora borreriæ Ell. and Ev.

Proc. Acad. Nat. Sci., Philadelphia, 1894, **46**, 379.

Chupp, C., *Monograph on the Fungus Cercospora*, Ithaca, N.Y., 1953, 491–92.

On living leaves of *Spermacoce hispida* (=*Borreira hispida*) (Rubiaceæ), Kottayam, 1–11–1956, T. S. R.

The infection appears as indistinct blotches extending over part or whole of the leaf, eventually turning light brown on the upper surface. On the corresponding lower surface effuse olivaceous growth of the fruiting bodies is evident. The conidiophores are multiseptate, geniculate, curved or tortuous and borne on stromata filling the stomatal opening. The conidia are subhyaline to pale olivaceous, narrowly obclavate and straight or slightly curved.

Cercospora caladii Cke.

Grevillea, 1879, **8**, 95.

Chupp, C., *Mongor. of the Fungus Cercospora*, 1953, p. 57.

On living leaves of *Colocasia antiquorum* Schott (Aroideæ), Kottayam, 10-9-1956, T. S. R.

Circular leaf-spots with grey centre and reddish-brown margin are formed on the leaves. Fruiting bodies are present on both surfaces, emerging through the stomata. The conidiophores are unbranched, septate, geniculate with dark spore scars. The conidia are hyaline, septate, broader at the base and tapering towards the apex.

C. colocasiæ Chupp (= *C. caladii* var. *colocasiæ* V. Hohnel) has been recorded on this host. But the conidia are coloured in this species while in *C. caladii* they are hyaline. Hence the fungus under study has to be included under *C. caladii*.

Cercospora carbonacea Miles

Trans. Ill. Acad. Sci., 1917, **10**, 255.

Chupp, C., *Monogr. of the Fungus Cercospora*, 1953, p. 196.

On living leaves of *Dioscorea alata* L. (Dioscoreaceæ), Kottayam, 12–9–1956, T. S. R.

The leaf-spots are conspicuous, angular and dark brown to almost black. The conidiophores are mainly hypophyllous, multi-septate, brown and unbranched. The conidia are curved, olivaceous and septate.

Cercospora dioscoreæ Ell. and Martin

Am. Nat., 1818, **16**, 1003.

Chupp, C., *Monogr. of the Fungus Cercospora*, 1953, p. 197.

On living leaves of *Dioscorea alata* L. (Dioscoreaceæ), Kottayam, 15–10–1956, T. S. R.

Large dark brown blotches surrounded by yellowish areas are evident on the upper surface of the leaves. On the lower surface olivaceous growth due to the development of the clusters of conidiophores is evident. These are septate and brown in colour. The conidia are subhyaline and indistinctly septate.

Cercospora fukushiayana (Matsumura) Yamamoto

Chupp, C., *Monogr. of the Fungus Cercospora*, 1953, p. 78.

On living leaves of *Impatiens chinensis* L. (Balsamineæ), Kottayam, 10-9-1956, T. S. R.

Amphigenous circular or sometimes irregular depressed spots, 0·30–1·50 cm. in diameter, are formed on the leaves. Several spots may coalesce to form large patches. The centre of the spot is light to dark grey or brown while the margin is distinctly brown. Clusters of conidiophores are found on both sides of the spots. The conidia are hyaline and slender, 45–70× 2·5 μ, with 3–5 indistinct septa.

The disease is common during the rainy season. In some instances defoliation takes place. Generally the disease appears when flowering commences. To begin with, the spot appears as an indefinite yellow patch. Later it becomes round, depressed and brown.

Cercospora perfoliata Ell. and Ev.

J. Mycol., 1889, **5**, 71.

Chupp, C., *Monogr. of the Fungus Cercospora*, pp. 152–53.

Syn. *C. agerati* Stevens

On living leaves of *Ageratum conyzoides* L. (Compositæ), Mundakayam, (Kerala), 20-9-1956, T. S. R.

Indefinite, circular spots, light yellow on the upper surface and whitish to grey and powdery on the lower surface are formed on the leaves. In the initial stages the infection has a superficial resemblance to spots formed by powdery mildew. The conidiophores are found predominantly on the lower surface. They emerge in small clusters through the stomata or are formed on the creeping effuse superficial hyphæ. The conidia are hyaline to subhyaline, 2-4 septate and cylindric to obclavate.

The infection is common in October and November and is widespread in many districts.

Exosporium bryophylli sp. nov.

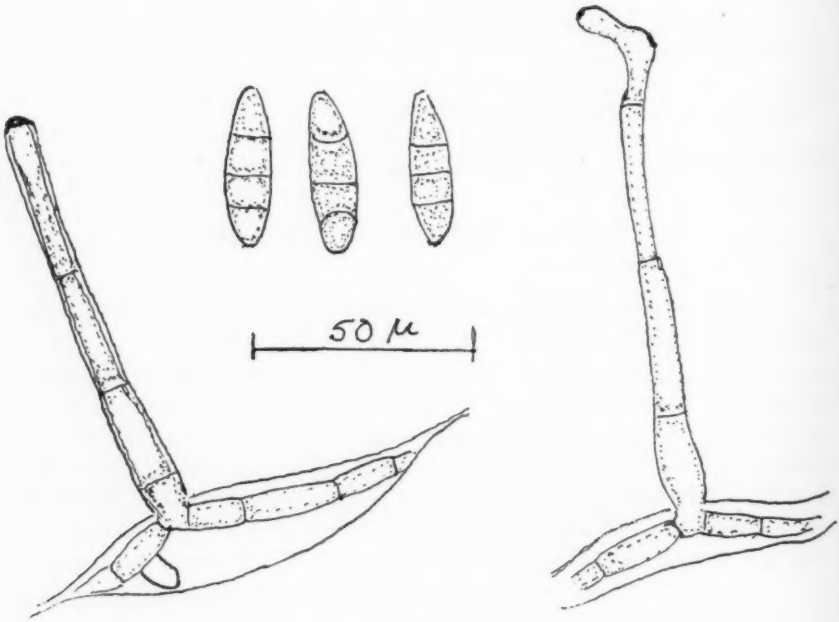
Spots circular to irregular, brown with black dense clusters of conidiophores on both sides; hyphæ internal collecting beneath the cuticle; conidio-

phores erumpent, dark brown, 3–4 septate, usually unbranched, measuring 140–285 × 6–7 μ; conidia acrogenous, spindle-shaped or obclavate, 3-septate, dark brown, 28–35 × 8–10 μ.

Maculæ circulares vel irregulares, brunneæ, fasciculis densis conidiophororum ad utrinque latus; hyphæ internæ, aggregatæ sub cutem; conidiophori erumpentes, fusce brunnei, 3–4 septati, ut plurimum haud ramosi, magnitudinis, 140–285 × 6–7 μ; conidia acrogena fusiformia vel obclavata, 3-septata, fusce brunnea, 28–35 × 8–10 μ.

On living leaves of *Bryophyllum calycinum* (Crassulaceæ), Muvattupuzha (Kerala), 15-8-1956, T. S. R.

The infection is evident only during the rainy months. The spots are prominent, sometimes depressed, with the conspicuous black dense clusters of conidiophores. Necrosis extends far beyond the region bearing the conidiophores.



TEXT-FIG. 2. *Exosporium bryophylli*—Conidiophores and conidia.

I am grateful to Rev. Dr. Santhapau for having kindly supplied the Latin translation.

THE RELATION BETWEEN LOSS IN VIABILITY AND SEED-BORNE MICRO-FLORA IN RICE

By S. Y. Padmanabhan

Received March 5, 1957
(Communicated by Sri. R. L. M. Ghose, F.A.SC.)

Introduction

When rice seeds are stored under wet and humid conditions, there is an increase in their moisture content and they rapidly lose their viability. It is generally held that such a loss in viability is brought about by the activity of moulds, but precise data to show the relationship between loss in viability and mould activity are lacking. Therefore, an investigation was carried out to determine the relationship, if any, between loss in viability of rice seeds under moist conditions of storage and mould activity.

Review of Literature

According to Johnson (1947) moulds spores found on surface of grains germinate and grow at critical moisture levels of the grains, particularly when air temperatures are warm; the fungi attack the carbohydrate, fats and proteins of grains by means of enzymes they secrete, resulting in raise of fat acidity, deterioration in quality and decrease in germinability. Loss in viability accompanied by increase in fat acidity was observed by Carter and Young (Johnson, *loc. cit.*) in wheat seeds but moulds were apparently not involved in this phenomenon. Crocker and Barton have reported (1953) that cotton seeds exposed to high moisture in field or to high moisture and temperature in storage showed marked correlation between the percentage of free fatty acid in the seed and viability. Sahadevan (1953) concluded that surface-borne moulds could not be the cause of deterioration in viability of rice seeds, since seeds treated with Agrosan GN also lost their viability in storage. On the contrary, Ramiah and Padmanabhan (1948) and Padmanabhan (1956) have shown that some fungicides like Cuprocide, Phygon and Spergon do help in preserving the viability of rice seeds stored under humid conditions.

Materials and Methods

Experimental.—Seeds of rice varieties were stored over different levels of relative humidity at room temperature. Over some of the levels the seeds lost their viability. The changes taking place in the moisture content, in germinability and the mould population carried in the seeds were recorded at periodical intervals. The investigation was carried out during the period, April–November, 1949.

The seeds of the following five varieties, T. 90, T. 812, T. 412, T. 1145 and T. 1242 were thoroughly sun-dried and their moisture percentage determined by standard methods. A sample of four oz. of seed of each variety was stored in May 1949 in large desiccators maintaining approximately 10, 25, 50, 75, 90 and 100% relative humidity levels over sulphuric acid dilutions. Fortnightly readings on the moisture content of the seeds were made till the end of July but afterwards the readings were taken only once a month. One hundred seeds of known moisture content were weighed and stored separately in the desiccators in small glass specimen tubes covered with muslin cloth. Variations in the moisture content of the seeds of each variety were calculated from the variations in the weight of the 100 weighed seeds determined at the stated intervals.

The germination percentage of the sun-dried seeds was nearly 100% in all cases before storage.

Four observations were taken on the germinability of the seeds during storage on:

6–6 to 12–6,
9–7 to 13–7,
4–8 to 7–8,
24–9 to 3–10.

The germination of the seeds were recorded on duplicate samples of 100 seeds drawn from the desiccators.

Isolation experiments to determine the nature and the type of microflora present internally were carried out on 5/5 to 16/5, (just prior to storage), 7/6 to 16/6, 19/7 to 27/7, 9/9 to 14/9 finally on 18/11 to 22/11. The determination of internally borne microflora were carried out with a sample of 100 seeds. The seeds were washed thoroughly in 10 ml. of sterile water, left to stand in the water for an hour, washed in a single change of sterile water, surface sterilised for a minute and a half in $HgCl_2$ of 1 : 1000 strength, washed once again in four changes of sterile water, and sown in thin Oatmeal agar plates. A week after sowing observations were recorded on the germination of the sterilized seeds and then fungus or fungi, if any, growing from the seeds were identified.

RESULTS

1. *Change in moisture content of seeds.*—The moisture contents of the sun-dried seeds of the five varieties were 13·5, 13·0, 12·5, 11·5 and 10% respectively, in T. 90, T. 812, T. 412, T. 1242 and T. 1145. Within a fort-

night of storage over the different R.H. levels when the first reading was taken the moisture content of the seeds attained equilibrium with the R.H. of storage. Further changes recorded were not large. When stored over 10, and 25% R.H. levels, the moisture content decreased in all varieties. Over 50% R.H. level also, there was a slight fall in the moisture content in the seeds of all varieties except T. 90, in which there was a slight increase. Over 75, 90 and 100% R.H. levels, the moisture content of the seeds of all varieties increased appreciably. The maximum increase recorded was in T. 90 (Table I).

2. *Relation between moisture content and germinability.*—As discussed more fully below, it may generally be said that increase in the moisture content of sun-dried seeds is sooner or later followed by deterioration in the germinability (Table I).

3. *Relation between loss in germinability and mould activity.*—There was no loss in viability of seeds stored over 10, 25, 50% R.H. levels. Over 75, 90 and 100% R.H. levels, loss in viability was seen to occur in all varieties sooner or later during the seven months of storage. Over 75% R.H. loss in viability was noticed in July test, two months after storage in T. 90, in August in T. 812, and in September only in other varieties. No moulds were recovered from any seed either in the second, third or even in the seventh month, when the seeds of some varieties had deteriorated appreciably (Table II).

Over 90% R.H. level, loss in viability was seen in June in T. 90 and in July in the other four varieties, but most prominently in T. 812. In the isolation test made in November there was no viable seeds left in any variety. Still no moulds were recovered from the seeds in any of the four isolation tests made in the second, third, fifth and seventh month of storage (Table III).

Over 100% R.H. level the seeds had deteriorated considerably even by the end of the first month of storage. In the third month there was practically no viable seed in any variety. But in the isolation tests made, no moulds were present in the seeds. In the isolation test made in the fifth month of storage, a couple of months after loss in viability had been recorded, *Penicillium* sp. could be isolated from 42, 34, 26, 20 and 4% of seeds of T. 812, T. 1242, T. 1145, T. 90 and T. 412, respectively. In the last isolation test made in the seventh month of storage, all the seeds tested were found to be colonised by *Penicillium* sp. (Table IV).

The isolation tests showed that there was an internal flora of a few species of fungi carried in almost all rice seeds (Padmanabhan, 1949). The population of internal flora remained unaltered during storage over 10, 25 and 50 per cent. R.H. levels (Table V, for results obtained over 10% R.H. of storage), *i.e.*, in the seeds which did not lose their viability. In the seeds

TABLE I

Showing the changes taking place in the moisture contents of sun-dried rice seeds when stored over different humidity levels during May–October 1949 *and the germination per cent of the seeds during the same period recorded in duplicate sets of* 100 *seeds each*

Month of testing	Germination per cent and moisture content (percentage) of seeds during storage over different relative humidity levels from May–October 1949					
	Relative humidity of storage					
	10 per cent	25 per cent	50 per cent	75 per cent	90 per cent	100 per cent
	T. 90 (*Original moisture content*—13·5 *per cent*)					
June ..	93·5	96·0	97·0	92·0	78·0	21·0
July ..	89·5	95·0	95·0	28·0	32·0	2·0
August ..	87·0	92·0	93·0	4·0	7·0	0·0
October ..	87·0 (10·1–10·9)	93·0 (10·8–11·4)	89·0 (13·9–14·4)	0·0 (16·3–16·8)	0·0 (16·9–17·4)	0·0 (18·8–19·8)
	T. 812 (*Original moisture content*—13·0 *per cent*)					
June ..	99·0	97·0	96·0	97·0	94·0	12·0
July ..	99·0	97·0	98·0	88·0	39·0	0·0
August ..	98·0	95·0	94·0	48·0	5·0	0·0
October ..	97·0 (9·7–10·8)	94·0 (10·1–11·1)	96·0 (12·0–12·4)	19·0 (14·0–14·7)	0·0 (14·6–15·3)	0·0 (16·8–17·9)

T. 412 (*Original moisture content*—12·5 *per cent*)

June	..	98·5	97·0	96·0	99·0	96·0	51·0
July	..	100·0	99·0	96·0	99·0	88·0	27·0
August	..	98·0	98·0	97·0	93·0	65·0	0·0
October	..	97·0	95·0	97·0	77·0	24·0	0·0
		(9·4–10·4)	(10·0–10·7)	(11·7–12·1)	(14·0–14·5)	(14·5–15·2)	(16·8–17·8)

T. 1242 (*Original moisture content*—11·5 *per cent*)

June	..	97·0	93·0	97·0	95·0	98·0	66·0
July	..	95·0	98·0	95·0	95·0	84·0	25·0
August	..	95·0	96·0	95·0	83·0	47·0	0·0
October	..	95·0	95·0	95·0	62·0	6·0	0·0
		(8·2–9·3)	(8·6– 9·5)	(10·4–10·9)	(12·7–13·3)	(13·6–14·2)	(15·9–17·0)

T. 1145 (*Original moisture content*—10·0 *per cent*)

June	..	99·0	97·0	96·0	99·0	99·0	44·0
July	..	99·0	99·0	100·0	100·0	91·0	10·0
August	..	99·0	97·0	94·5	97·0	58·0	0·0
October	..	97·0	100·0	99·0	85·0	6·0	0·0
		(6·8–7·7)	(7·2–8·1)	(8·1–8·5)	(11·3–11·9)	(11·8–12·5)	(14·3–15·4)

Note.—Figures in brackets indicate variation in moisture content during storage.

TABLE II

Showing the germination and the fungi isolated in 4 isolation tests made with seeds of 5 varieties of rice stored over 75% relative humidity level during 7 months of storage

Storage period in months	Germination per cent of seeds	Percentage of seeds observed with fungi	Total No. of fungi isolated from 100 seeds	*Trichoconis padwickii*	Other fungi†	Unidentified species*	Remarks
T. 90 (*Original moisture content*—13·5 *per cent*; *moisture content during storage*—16·3–16·8 *per cent*)							
2	92	56	66	32	14	20	Out of 80 seeds only
3	28	63	68	58	7	3	
5	4	..	..	..	..	..	No fungus growth; seedlings collapsed and died after germination
7	0	10	10	6	4	..	..
T. 812 (*Original moisture content*—13·0 *per cent*; *moisture content during storage*—14·0–14·7 *per cent*)							
2	98	100	100	78	4	18	
3	50	100	100	100	..	..	
5	16	48	48	24	..	24	
7	0	36	36	32	2	2	
T. 412 (*Original moisture content*—12·5 *per cent*; *moisture content during storage*—14·0–14·5 *per cent*)							
2	96	100	102	70	12	20	
3	94	100	118	80	31	7	
5	..	..	..	..	..	..	
7	60	72	74	15	12	47	

7	60	72	74	15	12	47	

T. 1242 (*Original moisture content*—11·5 *per cent*; *Moisture content during storage*—12·7–13·3 *per cent*)

2	96	82	88	62	14	12	
3	92	100	100	45	..	55	Fungal growth from 55 seeds could not be indentified as plates became contaminated
5	44	36	36	36	..	..	
7	28	14	14	4	10	..	

T. 1145 (*Original moisture content*—10·0 *per cent*; *moisture content during storage*—11·3–11·9 *per cent.*)

2	100	86	87	52	26	9
3	100	100	112	82	14	16
5	64	84	88	60	12	16
7	38	54	54	34	6	14

* Unidentified fungi include growth from seeds which fall away from medium before the growth established themselves on the medium.

† Other fungi include *Helminthosporium oryzæ, Curvularia lunata, Pseudocercospora* sp., *Cephalosporium* species.

TABLE III

Showing the germination and fungi isolated in 4 isolation tests made with seeds of 5 varieties of rice stored over 90% R.H. level during 7 months of storage

Storage period in months	Germination per cent of seeds	Percentage of seeds observed with fungi	Total No. of fungi isolated from 100 seeds	*Trichoconis padwickii*	Other fungi†	Unidentified species*
T. 90 (*Original moisture content*—13·5 *per cent*; *moisture content during storage*—16·9–17·4 *per cent*)						
2	80	80	83	75	8	2
3	12	34	34	32	..	..
5	0	22	22	18	2	2
7	0	20	24	16	6	..
T. 812 (*Original moisture content*—13·0 *per cent*; *moisture content during storage*—14·6–15·3 *per cent*)						
2	92	94	94	66	6	22
3	10	84	84	84	..	..
5	0	70	70	68	..	2
7	0	90	90	90	..	..
T. 412 (*Original moisture content*—12·5 *per cent*; *moisture content during storage*—14·5–15·2 *per cent*)						
2	94	100	106	56	26	24
3	85	100	100	70	3	27
5	8	36	36	28	2	6
7	0	56	56	54	..	2

T. 1242 (*Original moisture content*—11·5 *per cent* ; *moisture content during storage*—13·6–14·2 *per cent*)

2	98	100	100	46	22	32
3	58	85	86	48	16	22
5	2	56	56	36	6	14
7	0	32	24	2	4	2

T. 1145 (*Original moisture content*—10·0 *per cent* ; *moisture content during storage*—11·8–12·5 *per cent*)

2	100	84	84	56	4	24
3	72	92	94	70	10	14
5	6	46	46	44	2	..
7	0	42	42	36	..	6

* Unidentified fungi include growth from seeds which fall away from medium before the growths established themselves on the m.

† Other fungi include *Helminthosporium oryzae*, *Curvularia lunata*, *Pseudocercospora* sp., *Cephalosporium* species.

TABLE IV

Showing the germination and the fungi isolated in 4 isolation tests made with seeds of 5 varieties of rice stored over 100% R.H. level during 7 months of storage

Storage period in months	Germination per cent of seeds	Percentage of seeds observed with fungi	Total No. of fungi isolated from 100 seeds	*Trichoncois padwickii*	Other fungi†	*Penicillium* species	Unidentified fungi*
T. 90 (*Original moisture content*—13·5 *per cent* ; *moisture content during storage*—18·8–19·8 *per cent*)							
2	21	70	70	39	3	..	28
3	2	20	20	20	..	..	..
5	0	20	20	..	..	20	..
7	0	100	100	..	..	100	..
T. 812 (*Original moisture content*—13·0 *per cent* ; *moisture content during storage*—16·8–17·9 *per cent*)							
2	12	96	96	76	12	..	8
3	0	69	69	58	..	..	11
5	0	42	42	..	..	42	..
7	0	100	100	..	..	100	..
T. 412 (*Original moisture content*—12·5 *per cent*; *moisture content during storage*—16·8–17·8 *per cent*)							
2	51	97	97	55	16	..	26
3	27	60	60	45	..	..	15
5	0	4	4	..	..	4	..
7	0	100	100	..	..	100	..

T. 1242 (*Original moisture content*—11·5 *per cent* ; *moisture content during storage*—15·9–17·0 *per cent*)

2	60	80	84	56	10	..	18
3	5	38	38	38	..	..	..
5	0	34	34	..	..	34	..
7	0	100	100	..	..	100	..

T. 1145 (*Original moisture content*—10·0 *per cent* ; *moisture content during storage*—14·3–15·4 *per cent*)

2	44	82	82	60	6	..	16
3	10	18	18	15	..	..	3
5	0	26	26	..	..	26	..
7	0	100	100	..	..	100	..

* Unidentified fungi include growth from seeds which fall away from medium before the growths established themselves in the medium.

† Other fungi include *Helminthosporium oryzæ*, *Curvularia lunata*, *Pseudocercospora* sp., *Cephalosporium* species.

TABLE V

Showing the germination and the fungi obtained in 4 isolation tests made with rice seeds of 5 varieties stored over 10% R.H. level during 7 months of storage (similar results were obtained with 25 and 50% R.H. levels)

Storage period in months	Germination per cent of seeds in isolation tests on sterile media	Percentage of seeds observed with fungi	Total No. of fungi isolated from 100 seeds	*Trichoconis padwickii*	Other fungi‡	Unidentified fungi*
T. 90 (*Original moisture content*—13·5 *per cent* ; *moisture content during storage*—10·1–10·9 *per cent*)						
2	87	53†	55	41	16	8
3	88	100	102	82	12	8
5	80	74	76	48	4	24
7	88	88	90	82	4	4
T. 812 (*Original moisture content*—13·0 *per cent* ; *moisture content during storage*—9·7–10·8 *per cent*)						
2	96	86	92	76	12	4
3	99	100	106	92	12	2
5	..	..	..	..	..	..
7	88	100	102	90	12	..
T. 412 (*Original moisture content*—12·5 *per cent* ; *moisture content during storage*—9·4–10·4 *per cent*)						
2	100	100	100	70	30	..
3	96	100	120	82	12	26
5	..	..	..	..	..	..
7	92	98	98	82	16	..

T. 1242 (*Original moisture content*—11·5 *per cent* ; *moisture content during storage*—8·2–9·3 *per cent*)

2	98	89	96	56	32	8
3	100	100	116	66	24	26
5	100	95	105	53	30	22
7	96	100	108	84	18	6

T. 1145 (*Original moisture content*—10·0 *per cent* ; *moisture content during storage*—6·8–7·7 *per cent*)

2	98	89	89	62	12	15
3	100	98	99	68	2	29
5	..	..	..	..	..	..
7	96	100	108	84	18	6

---

* Unidentified fungi include growth from seeds which fall away from medium before the growths established themselves on the medium.

† Out of 80 seeds only.

‡ Other fungi include *Helminthosporium oryzæ, Curvularia lunata, Pseudocercospora* sp., *Cephalosporium* species.

stored over 75, 90 and 100 % R.H. the flora gradually failed to appear in the seeds which had lost their viability (Tables II, III and IV).

DISCUSSION AND CONCLUSIONS

Loss in viability of rice seeds was found to follow absorption of moisture by the sun-dried seeds during storage. During the first three months of storage no moulds could be isolated from any of the seed samples, though deterioration in viability had set in from the first month in some cases. At the end of the seventh month when there was a considerable deterioration in the seeds of all varieties over 75 and 90% R.H., no moulds were found in the seeds stored over these levels. Over 100% R.H. there was practically no germination in the seeds after three months of storage, but in the isolation test made during this period, no moulds were present in the seeds. However, in the test made in the fifth month, 4% of seeds in T. 412, 20% in T. 90, 26% in T. 1145, 34% in T. 1242, and 42% in T. 812 yielded moulds on isolation. In the last test moulds could be isolated from all the seeds tested. Therefore, it may be concluded that the moulds began colonising the seeds between the third and fifth month of storage long after the germinability of the seeds were lost due to other causes. Loss in viability had preceded mould colonisation in the seeds stored over 100% R.H.

The normal internal flora also deteriorated along with the embryo when stored over high relative humidity levels.

Thus under high relative humidity of storage, loss of viability sets in gradually after absorption of moisture by rice seeds without the intervention of moulds. Increase in fat acidity is known to follow absorption of moisture by seeds (Carter and Young, *loc. cit.*, and Crocker and Barton, *loc. cit.*). Under normal germination in the presence of sufficient water, fatty digestion goes on *pari-passu* with increased metabolism of the growing embryo but when only lipolytic and other enzymatic activity are stimulated by increased moisture content without corresponding utilisation, the accumulation of fatty acid, etc., may prove toxic to the embryo. Further detailed investigations on the biochemical changes which take place inside the rice seeds following absorption of moisture under high humidity levels are necessary to throw more light on loss in viability of rice seeds over humid conditions of storage.

Only a few fungicides are effective in preserving the viability of rice seeds under humid storage condition. Whether the few effective fungicides interfere with the biochemical and enzymatic activity set up inside rice seeds when they absorb moisture has to be investigated to obtain an understanding of this aspect of the problem.

The experiment was carried out with small samples in the laboratory. Under bulk storage, the conditions and factors influencing deterioration may be somewhat different.

Summary

The changes taking place on the population of fungi internally borne in rice seeds during storage over relative humidity levels of approximately 10, 25, 50, 75, 90 and 100% were studied. Over 10, 25, and 50 % relative humidity levels there was a decrease in moisture content, but there was no deterioration in germinability within the period studied.

During storage over relative humidity levels of 75, 90 and 100% there was deterioration in germinability of seeds following increase in their moisture content.

No moulds could be isolated from the seeds stored over 75 and 90% relative humidity even after they had lost their vaibility during the course of the tests. Moulds were, however, isolated from seeds which had lost their viability during the storage over 100% R.H. level.

Deterioration in germinability was followed by decrease and disappearance of the normal internally borne fungi and this was followed by colonisation of the rice grain by principally *penicillia* in the case of seeds stored over water (*i.e.*, over 100% R.H.).

It is concluded that mould activity has no direct relation to the inactivation of embryo in rice seeds in storage under wet and humid conditions.

Acknowledgements

The author wishes to acknowledge the useful suggestions given by Mr. R. L. M. Ghose, Director, Central Rice Research Institute, Cuttack, in writing the paper.

Literature Cited

1. Crocker, W. and Barton, L. V. *Physiology of Seeds*, Chronica Botanica Coy., 1953, 44.
2. Johnson, A. G. .. *The Role of Fungi in the Deterioration of Grains in Starage*, 1947.
Preservation of Grains in Storage, F.A.O. Publication, Washington, 1948, 88–92.
3. Padmanabhan, S. Y. .. "Occurrence of fungi inside rice kernels," *Curr. Sci.*, 1949, **18**, 442–43.
4. ——— "Preserving viability of rice seeds with fungicides," *Indian Phytopath.*, 1956, **9**, 44–47.
5. Sahadevan, P. C. .. "Studies on the problem of loss of viability of rice seeds in storage," *Madras Agric. Journ.*, 1953, **40**, 133–43.
6. Ramiah, K. and Padmanabhan, S. Y. "Seed treatment of Rice," *Proceedings of 36th Session of Indian Science Congress, Agricultural Science*, 1949 (Abs.).

STUDIES ON THE EGG MASSES AND LARVAL DEVELOPMENT OF SOME PROSOBRANCHS FROM THE GULF OF MANNAR AND THE PALK BAY*

By A. V. Natarajan

(Government of India Senior Research Scholar in Marine Biology, Central Marine Fisheries Research Station, Mandapam, S. India)

Received April 8, 1957

(Communicated by Dr. N. K. Panikkar, F.A.SC.)

CONTENTS

		Page
1.	Introduction	170
2.	Material and Methods	172
3.	Description of the Eggs and Larvæ of Some Prosobranchs	173
4.	Discussion	219
5.	Summary	223
6.	References	223

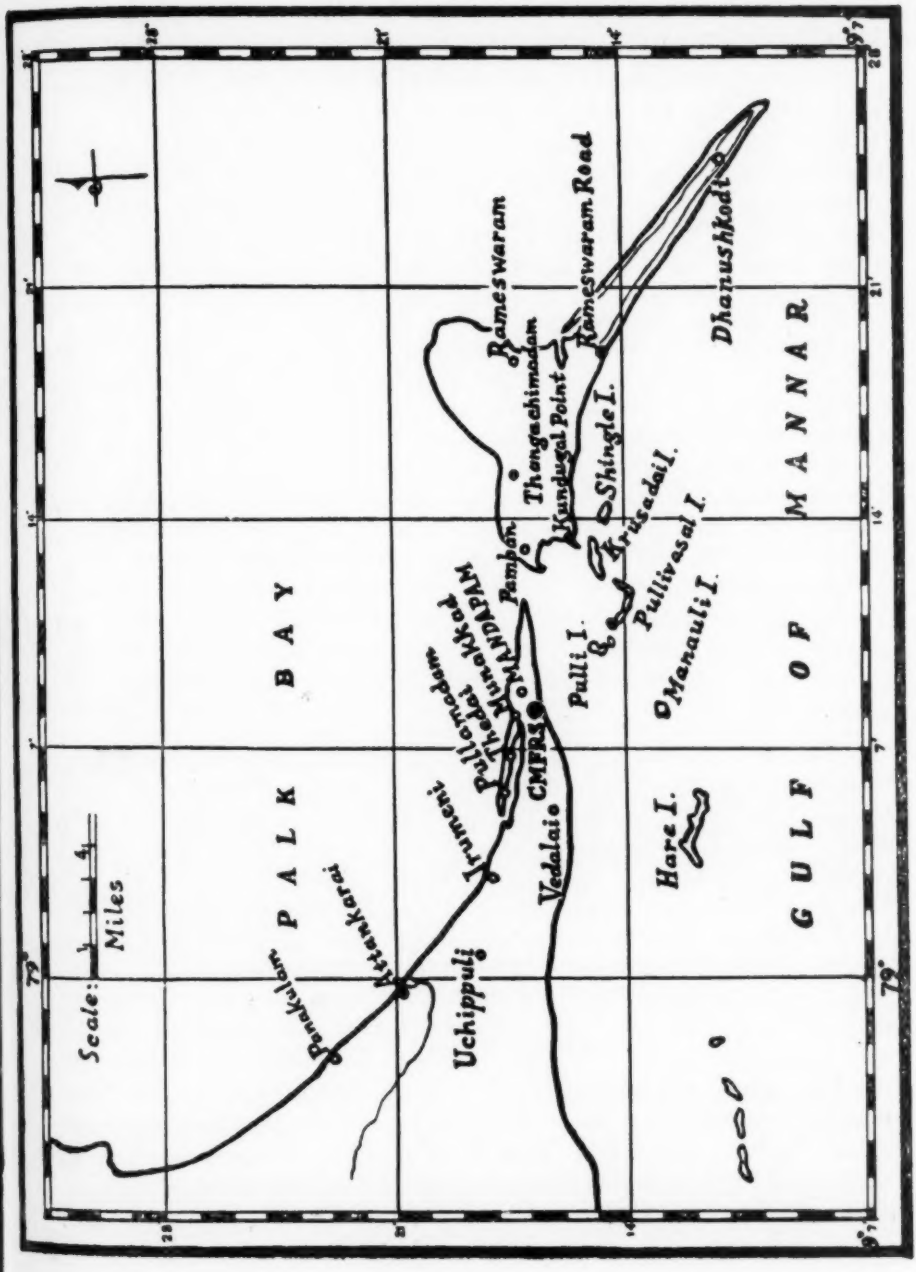
1. Introduction

In recent years our knowledge of the eggs and larvæ of marine gastropods has increased considerably. The investigations of Lebour (1937) and Thorson (1946) have added much to our knowledge of the eggs and larvæ of prosobranchs from Plymouth and Danish waters. Franc's (1948) investigations at Algiers and Banyuls supplement at many points the work of Lebour and Thorson. The breeding habits of this group of molluscs at Bermuda as well as in East Greenland and Iceland have also been studied respectively by Lebour (1945) and Thorson (1935, 1941).

In the Indo-Pacific area the breeding habits, egg masses and larval development of some prosobranchs have been studied by Risbec (1928, 1931, 1932, 1935) and Petit and Risbec (1929) from the New Caledonian coast, by Thorson (1940) from the Iranian Gulf, by Ostergaard (1950) from the Hawaiian coasts, and Habe (1944, 1953, 1955) and Amio (1950) from the Japanese waters. Lamy (1928) has summarised the older literature on the

* From the *Thesis* approved for the degree of Master of Science of the Annamalai University.

Published with the permission of the Chief Research Officer, Central Marine Fisheries Research Station, Mandapam Camp, S. India.



MAP 1. Showing vicinities of Mandapam wherefrom the prosobranch egg masses were collected.

tropical prosobranch egg masses and Knudsen (1950) described a large number of them from West Africa.

No concerted attempt appears to have been made so far from any locality in India to study the breeding habits of the marine gastropod molluscs. Hornell (1921) has made casual reference to eight egg masses and Gravely (1942) to twelve, some of which have not been ascribed to any definite species. A few details are also available on the egg masses of *Thais carinifera* (Annandale and Kemp, 1916), *Potamides cingulatus* and *Natica* (Panikkar and Aiyar, 1939), *Tonna* sp. (Panikkar and Tampi, 1949) and *Thais bufo* (Chari, 1950) and on larval development of *Xancus pyrum* (Chidambaram and Unny, 1947).

From the above résumé it is clear that studies on the breeding habits and the larval development of marine prosobranchs have not received much attention from Indian workers. Krusadai, Pamban and Mandapam are well known for their rich molluscan fauna and it was suggested to me that the breeding biology of them will be of considerable scientific interest. The present work may be said to follow broadly the pattern of investigations of Risbec (1931, 1932, 1935) from New Caledonia, Thorson (1940) from Iran, Rasmussen (1944, 1951) from Denmark, Lebour (1945) from Bermuda, Knudsen (1950) from West Africa and Ostergaard (1950) from Hawaii. Thiele's *Handbuch der systematischen Weichtierkunde* (Jena, 1931) and Satyamurti's (1952) *Mollusca of Krusadai Island* have been generally followed for systematic classification and nomenclature of the forms dealt with in this paper.

The work reported here is the result of the investigations of over three years from 1952–55 carried out at the Central Marine Fisheries Research Station, Mandapam, S. India. The writer expresses his gratitude to Dr. N. Kesava Panikkar for suggesting this problem as well as for his guidance, suggestions and advice throughout the course of this work. Thanks are also due to Shri K. Virabhadra Rao for his willing help and suggestions during the progress of this investigation; to the Government of India for the award of a scholarship; and to Shri K. G. Nambiar for the photographs included in this paper.

2. MATERIAL AND METHODS

The material was collected from the Gulf of Mannar and the Palk Strait at different places, *viz.*, Krusadai Island, Shingle Island, Pulli Reef, Kundugal Point, Pamban, Munakkad, Vedalai, Seeniyappa Dharuga, Mandapam, Rameswaram Road and Pudumadam (Map 1). The material examined comprises spawn masses, embryos and free swimming veligers of 32 prosobranchs,

Plankton samples from the Palk Bay and the Gulf of Mannar were examined periodically. The spawn masses are usually found on the undersurfaces of the stones, boulders or upon the algæ, dead shells, corals and crevices of the rocks. The mud-flats at Kundugal Point form an ideal substratum for the characteristic egg ribbons of *Natica*. Some of these spawn masses themselves serve for the attachment of the spawn of others. The adult animals were kept in aquaria to observe the spawning habits. This also helped in corroborating the provisional identification of the egg masses obtained from natural habitats. Breeding season was determined as far as possible for each species under investigation.

Salinity and temperature were noted during spawning periods wherever it was possible. The chlorinity of samples of sea-water was estimated by the Mohr titration with the silver nitrate using potassium chromate as indicator and following an experimental procedure similar to the one described by Harvey (1945). By referring to Knudsen's tables (1901) the salinity, *i.e.*, parts by weight of sea-water was read off corresponding to the estimated chlorinity.

Magnesium salts were found quite useful in narcotising the larvæ for which the following procedure was followed. Filtered sea-water was taken in a medium sized watch-glass and a few larvæ were transferred to it. Saturated solution of magnesium chloride or magnesium sulphate (both were found equally effective) were added drop by drop at intervals of 4–5 minutes. Usually 3–7 drops were found sufficient to narcotise the larvæ. As soon as narcotisation was complete the larvæ were transferred to a cavity slide and sketched. Menthol was also found effective in some cases.

The material was mostly examined and sketched in the living condition. The larvæ were sketched with camera lucida and the measurements taken with the help of an ocular micrometer. The named collections, preserved in 5% formalin, are now kept in the museum of the Central Marine Fisheries Research Station, Mandapam Camp.

Various terms, egg ribbon, egg sheet, egg case, and egg cluster have been used in this paper to denote the spawns of the prosobranchs according to their characteristic features, but the term, egg mass, is used to denote all types.

3. Description of the Eggs and Larvæ of Some Prosobranchs

Neritidæ

The egg capsules of the genus *Nerita* have been described for a number of species (Lamy, 1928; Risbec, 1932, 1935; Andrews, 1935; Lebour, 1945;

Knudsen, 1950). It has been observed that the walls of the egg capsules in Neritidæ contain small mineral particles. In *Nerita* these particles were found to consist of spherulites of calcium. No account is available on the breeding habits of *Nerita* from Indian waters.

Nerita albicilla Linné (Figs. 1–6).—The egg capsules belonging to *Nerita* have been collected from Shingle Island attached to stones and dead corals in the month of February, April, May, July, August and September. The only species that appears to be common in the area examined is *N. albicilla* to which species therefore the present egg capsules are referred.

The egg capsules which are firmly glued to the substrata are white and hardy and are round or oval in shape. They are plano-convex, and each capsule consists of a membranous base and calcareous convex top or lid. The largest diameter of the capsule in 14 specimens examined is 1·87–2·48 mm. The lid consists of numerous cup-shaped structures (circular in outline), placed close to each other and each consisting of a spherical body inside.

The eggs, which are spherical and white, measure each 0·116 mm. across. They are suspended in a mucous fluid. The number of eggs per capsule is 121–85. In the present case it is not possible to give the time of development from the egg to the hatching stage. Risbec (1932) found that after 52 days the veligers of *N. albicilla* from New Caledonia were still unhatched but moving freely inside the capsule.

The newly hatched veliger has a shell of one whorl which is slightly pitted in appearance and measures 0·150–0·167 mm. across the shell. The velum is bilobed, colourless and is bordered with long cilia. Eyes are black and prominent. Tentacles could not be seen. Foot is ciliated and greenish. It also shows reddish brown pigments on either side at the base of the foot. Risbec finds vermillion pigments in the New Caledonian species. Operculum is present. Otocysts are not clear. Liver is pale yellow. The larva thrived at the laboratory for two days.

Potamididæ

Panikkar and Aiyar (1939) have given an account of the spawn of *Potamides cingulatus* (*Cerithidea fluviatilis*) from Madras and Habe (1955) that of *Cerithidea djadjariensis* and *C. rhizophorarum* from Japan.

Cerithidea fluviatilis (Potiéz and Michaud) (Photos 1 & 2 and Figs. 7–9).—No details appear to be available on the structure of the egg mass or larvæ of this species except for the observation of the spawn masses of this species at Madras by Panikkar and Aiyar. Sadasivan (1948) has made detailed studies on the rate of growth, the duration of the breeding period and the age at maturity of this species, also from Madras.

This species is very well represented at Kundugal Point wherein large areas are strewn with large numbers of live as well as dead shells, the latter quite commonly being occupied by hermit crabs. This species does not frequent the reefs exposed to the open surf. Satyamurti (1952) has also recorded them at Pamban and Krusadai Island.

The spawn masses of this species were found in the January-September period. Quite often they are found on the muddy substratum; some are attached to the inner or outer surface of the *Natica* egg ribbons as well as to dead or live shells, chiefly bivalves and seaweeds. The eggs are laid in narrow gelatinous strings of a width of 0·30–0·50 mm. The egg string is thrown into a series of more or less identical folds placed compactly side by side into a ribbon-like structure. The egg ribbons, in the old state, do not show the egg strings clearly. They are white and are of a width varying from 2·3–2·7 mm. and a length from 8·4–16·3 cm. They in turn may be thrown into folds, regular or irregular. The gelatinous part of the ribbon is colourless and transparent and the eggs are quite clearly seen through it. The egg is yellowish white and measures 0·133–0·150 mm. across. This is surrounded by a thin-walled egg covering (capsule) measuring 0·233–0·283 mm. The egg covering may be round or oval in outline. On an average an egg ribbon contains about 4,800 eggs.

Some of the adult specimens kept at the laboratory in a glass trough in mid-December 1953 spawned on the 10th January 1954. The spawn was glued to the side of the glass trough very near the surface of the water. The temperature and salinity at the time of spawning were 25·50° C. and 31·18‰ respectively.

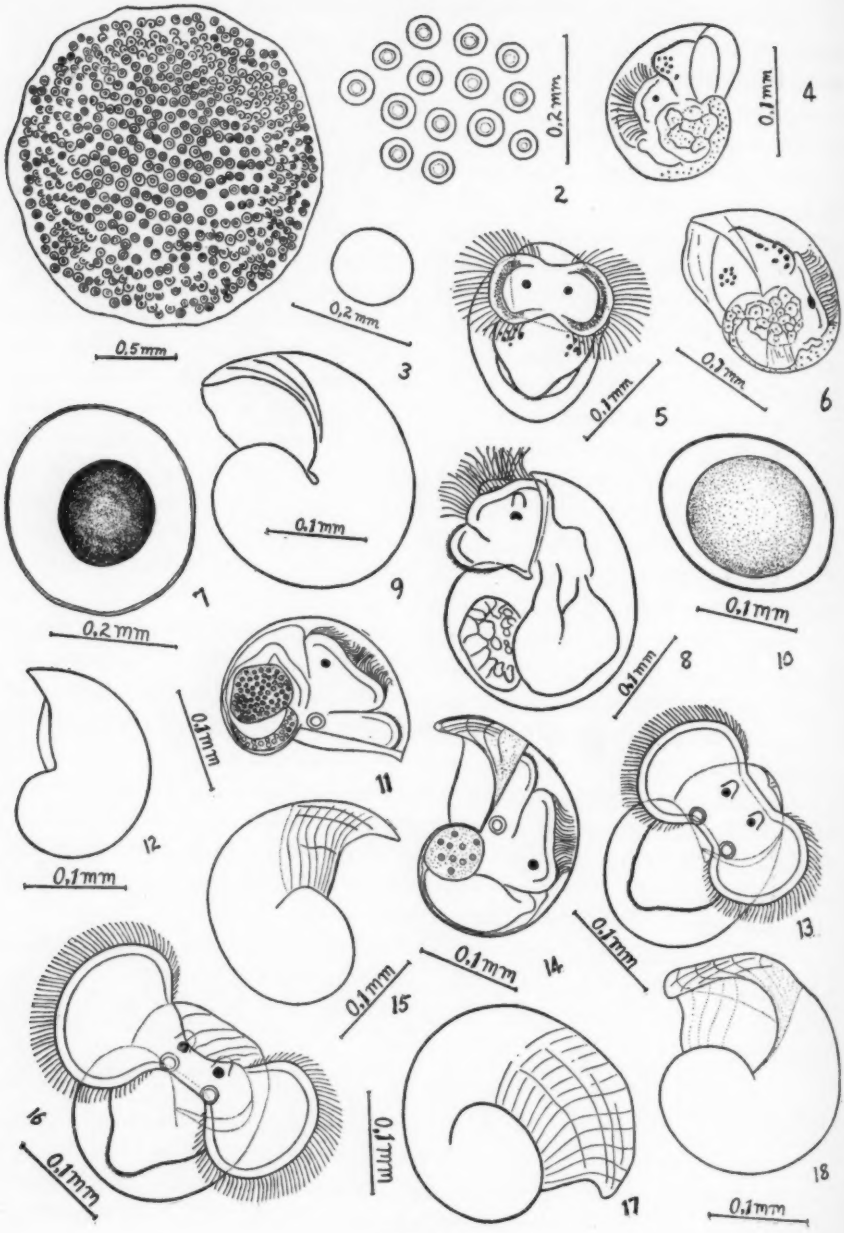
It took about 4 days from the time the eggs were laid for the larvæ to hatch. The embryo at the time of hatching measures about 0·200 mm. across the shell which is rough and semi-transparent. The outer lip of the shell, as soon as the embryo hatches out as veligers, begins to show transpiral striations and is slightly extended forward in the middle.

The umbilicus is slightly pinkish purple. The larva is pale yellow. The velum is thick and bilobed. The eyes, tentacles and otocysts are present and the foot is ciliated. The alimentary tract and liver are yellow. The heart beat could be seen through the shell.

The spawn masses collected from the field do not show normal development under laboratory conditions.

Cerithiidæ

The breeding habits of a few species of *Cerithium* are known in the literature (Lo Bianco, 1888; Risbec, 1935; Lebour, 1945). The members of the


0,5 mm
0,2 mm
2
0,1 mm
4
0,2 mm
3
0,1 mm
5
0,1 mm
6
0,1 mm
9
0,2 mm
7
0,1 mm
8
0,1 mm
10
0,1 mm
11
0,1 mm
12
0,1 mm
13
0,1 mm
14
0,1 mm
15
0,1 mm
16
0,1 mm
17
0,1 mm
18
0,1 mm

FIGS. 1-18

genus *Bittium* also appear to have spawns of similar nature (*cf.* Meyer and Mobius, 1872; Thorson, 1946).

Cerithium morus Lamarck (Photos 3 & 4 and Figs. 10–18).—The breeding habits of this species are described here for the first time. This species is abundantly represented on the reefs in the Palk Bay between Munakkad and Mandapam. Living and dead shells of this species are found quite commonly in Pamban in shallow areas and it appears to be a very prolific breeder during its spawning season. From the number of spawn masses collected in the field it is clear that the breeding starts about the middle of January, reproductive activity being somewhat low during January and February (the mean temperature and salinity for the months being 24·4° C., 27·41‰ and 24·7° C., 30·03‰ respectively). Large number of spawns have been encountered in March (mean temperature 27·1° C., mean salinity 32·74‰). It is only in April (mean temperature 28·6° C., mean salinity 33·69‰), May (mean temperature 27° C., mean salinity 34·39‰) and June (mean temperature 27·2° C., mean salinity 34·39‰) that the spawning is very active with the climax probably in May. There is a steep decline in spawning in July (mean temperature 29·9° C., mean salinity 34·8‰) which almost comes to an end by the last week of July. The species seems to have no preference for any specific substratum as the spawn masses have been collected from corals, stones, seaweeds, dead shells, etc.; they also spawn on the glass sides of the aquarium, glass troughs and even finger bowls. In the aquarium the spawn masses were laid close to the surface of water as well as the middle and the base of the glass sides. In some, part of the spawn mass is completely clear of the water, the rest being just immersed.

The eggs are laid in a long string of a width of 0·25–0·41 mm. The egg ribbon is formed of this egg string in much the same way as in *C. fluviatilis*. It is 60–185 mm. long, 1·72–2·75 mm. broad and 0·45 mm. thick and is surrounded by a gelatinous matrix, which is whitish or pale yellow in colour. The latter is quite transparent making the eggs visible. Two specimens were observed as soon as the spawning began. One of them (length 19 mm. and largest breadth 9 mm.) took about 2¼ hours to complete a ribbon of a length of 75 mm.; the other (length 18 mm. and largest breadth 8 mm.) took 1 hour and 50 minutes to complete 70 mm. An egg ribbon contains on an average 9,100 eggs. The disposition of the eggs in some ribbons are close while in others they are distantly placed. Thus two egg ribbons with similar dimensions may differ in their egg numbers. The eggs are yellowish white and measure each about 108–16 μ in diameter and are each surrounded by an egg covering of a diameter of 150–67 μ. The egg coverings are spherical, oval

or oblong in shape. They are flexible and transparent and usually do not touch each other and are placed at a distance of 12–18 μ from one another.

It takes just more than 3 days for the eggs to hatch out as free swimming veligers. The embryos in the hatching stage are yellow in colour and show smooth, colourless, sculptureless and fairly transparent shells each of which measures about 0·150–0·167 mm. across. The velum is bilobed and shows long cilia on the margin. Foot, operculum and otocysts are present. The liver is yellow.

The day-old veliger measures 0·200 mm. across the shell which shows slow changes to brown colour. The outer lip begins to show a few spiral striations besides the transpiral striations and is extended in the middle to form a beak-like structure. The umbilicus is slightly purplish in colour. The bilobed velum is well developed, but the lobes tend to show slight disparity in size. The velum measures about 0·200 mm. There is a sub-velum with small cilia. The eyes are black and distinct. The tentacles are stout, broad and well developed. The foot is pale yellow and is ciliated. The operculum and otocysts are present. The liver is pale yellow. The heart beat is visible.

The four-day old veliger shows further increase in the size of the larval shell. The latter is brown and measures about 0·217 mm. across. Spiral lines have become more prominent besides the transpiral striations. The outer lip of the shell is further extended and slightly inflexed. The velum now measures 0·288 mm. across. The disparity in lobes is also more pronounced. The sub-velum is more prominent. The larvæ lived at the laboratory for 5 days. The well-developed velum of the veliger points to a long pelagic period in its life-history.

Cerithium sp. (Photos 5 & 6 and Figs. 19–22).—Gelatinous white masses characteristic of *Cerithium* were found attached to stones, corals, shells, seaweeds, etc., in between tide marks at Munakkad besides those of *C. morus*. As the egg mass could not be referred to the adult with certainty it is described here as *Cerithium* sp.

The spawn masses were observed in January–July period. The eggs are laid in a long string which as in *C. morus* is folded upon itself closely and compactly and forms a ribbon. The width of the egg string is 0·133–0·200 mm. and that of the ribbon 0·835–1·00 mm. The ribbon, in its turn, may be thrown into loose or compact folds. Occasionally they are wavy or in a straight line. The spawn mass is covered by a slimy gelatinous matrix which is whitish or pale yellow in colour. Each egg is spherical, measures 0·066–0·083 mm. across, and is surrounded by an egg covering (capsule)

which measures 0·108 mm. across. The capsules are transparent and may or may not touch one another.

It takes about 4 days for the eggs to hatch out as free swimming veligers. The entire ribbon appears to dissolve away at the time of hatching. The newly hatched veliger measures 0·150 mm. across the shell which is unsculptured. The outer lip of the shell is slightly extended in the middle. The velum is bilobed, the lobes being of almost equal size. The eyes and tentacles are not yet developed. The foot is ciliated. The operculum is colourless and the otocysts are distinct. The liver is pale yellow.

The two-day old veliger shows further growth in the size of the larval shell which measures 0·167 mm. across. The projection in the middle of the outer lip is more pronounced. The tentacles and eyes are now developed and are clearly seen. The velum begins to show a sub-velum lined with small cilia. The retractor is visible. The veliger thrived at the laboratory for about 4 days. During that period no further changes were noticeable in the larva. However the well-developed velum comparing to the size of the larva points to a long pelagic period in its life-history.

Ianthinidæ

The egg cases of *Ianthina* have been described by several authors outside India (Lamy, 1928; Laursen, 1953; Wilson and Wilson, 1956). Hornell (1921), Crichton (1941) and Gravely (1942) have made brief references to the egg cases of the same genus from India.

Ianthina prolongata Blainville (Photos 7 & 8 and Figs. 23 & 24).—Brief references have been made to the egg cases of this species by Crichton, Gravely and Laursen (*l.c.*).

In the month of July and August, the period of strong winds which begin prior to the outbreak of south-west monsoon accompanied by rough seas, large number of floats of *Ianthina* with the egg cases attached underneath were collected along the shores of Gulf of Mannar near Vedalai. But during this period, in the present observation, floats were not found attached to the foot of the adult animals even though animals together with floats were reported. There appears to be only two species of *Ianthina* in Pamban area. They are *I. janthina* and *I. prolongata*. As the former appears to be viviparous, I am inclined to refer the present egg cases to the latter species.

Three floats with capsules in tact showed 110, 121 and 129 capsules respectively. Each capsule is a flattened, compressed pouch and is pale mauve in colour. In the preserved specimens (preserved in 5% formalin) the capsules become more or less colourless. The surface of the capsules is not

smooth but wrinkled appreciably. The sides of the capsule are in many cases wavy. The distal end of the capsule is prominently but promiscuously jagged. The jags appear in certain places, to be arranged in 6–9 rows. The sides are also jagged in some capsules but less prominently. The capsules are not of uniform shape but generally pyriform or oblong pyriform.

The capsules are suspended from underneath the float. Each capsule is provided with a brownish-white stalk (0·6–0·9 mm. long and 0·116 mm. wide), which is slightly twisted at the base. The length and breadth of the capsule vary from 4–6 mm. and 1–3 mm. respectively. The number of eggs per capsule ranges from 1,137–2,250. On an average each egg mass contains 220,000 eggs.

The eggs appear to be suspended in a white nutritive fluid. Each egg measures about 0·066 mm. The egg capsules kept at the laboratory on 21st August 1954 liberated the veligers on 23rd August 1954. At the time of hatching the entire top of the capsule slits open. Unfortunately the newly hatched veligers were attacked by the ciliates and were disturbed to a great extent. However, some of the less affected larvæ reveal the following details. The surface of the larval shell is rough and uneven when observed under high power. It measures 0·083–0·100 mm. across. The velum is more or less in the form of a sheet without any lobes. It is colourless and bordered with small cilia. Eyes and tentacles could not be seen. Foot and operculum are present but otocysts invisible. The internal organs are not clearly distinguishable except for the liver. The larvæ at this or later stage of development could not be traced in the plankton samples collected from the Gulf of Mannar.

Naticidæ

The characteristic egg mass of this family is well known for a considerable number of species from other parts of the world (Lamy, 1928; Ankel, 1930; Melville, 1930; Hertling, 1932; Thorson, 1935, 1940, 1946; Lebour, 1937, 1945; Knudsen, 1950; Giglioli, 1955). Very little is known about the breeding habits of *Natica* from India except for brief references by Hornell (1921), Panikkar and Aiyar (1939), Gravely (1942) and Satyamurti (1952).

Natica marochiensis Gmelin (Photo 9 and Figs. 25–30).—This is the commonest species of *Natica* collected from the Pamban area. Large numbers of live specimens have been collected from Kundugal Point in certain areas on the mud flat.

Besides Gravely and Satyamurti who have made brief references to the egg ribbons of this species from Madras and Kundugal Point respectively,

the egg ribbon of this species has also been recorded from West Africa by Knudsen (*l.c.*).

The mud flats at Kundugal Point have been strewn with enormous numbers of egg ribbons of this species. The adult gastropods are not so commonly seen as their egg ribbons. The egg ribbons were also observed at Vedalai but no adults could be found in that area. Some of the adults of the present species were kept in the glass troughs with sandy bottom of 1½–2″ depth. They laid the egg ribbons in the month of January, temperature and salinity being 25·2° C. and 27·12‰ respectively. The egg masses were observed in the field in the months of January, February, March, April, May, July, August, September, November and December. The breeding appears to take place all the year round.

The egg ribbons are in the form of an open ring, sometimes thrown into a spiral of 1½–2 rounds. They are encrusted with sand particles. They are usually greyish in colour. These sand agglutinated egg ribbons form fine substrata for *Cerithidea fluviatilis* which lay their gelatinous spawn masses not infrequently on their inner or outer surfaces. Eight egg ribbons taken at random for examination measure 3·5–5·0 cm. across. They are about 81–190 mm. long (measured along the periphery), 10–13 mm. broad and 0·45–0·52 mm. thick. The egg ribbon of the West African species is about 117 mm. in length (measured along the periphery) and the innermost half of the egg ribbon is protruded as a broad collar giving the egg mass a somewhat conical shape. In the Indian species the 'collar' of the ribbon is not distinguishable. The inner margin of the egg ribbon is even and regular. So is the periphery though it is sometimes undulated or thrown into minute folds. The average number of egg spaces per row as seen in the vertical trans-section (*i.e.*, internal view of the egg structure as seen in the cross-section by flattening the egg ribbon and cutting across its width) is 24 and there are about 122 such rows lengthwise (*i.e.*, the length of the egg ribbon when it is uncoiled and flattened out on a plane surface). The egg spaces are absent along the periphery and inner margin. Roughly there are about 2,900 egg spaces per egg ribbon, each egg space measuring 0·183–0·367 mm. across. It contains 3–7 eggs. Each egg is surrounded by a thin and transparent egg capsule (egg covering). The egg and egg covering measure across 0·066–0·083 mm. and 0·133 mm. respectively. Knudsen's account does not contain any of these details except that there are 20–25 embroys per egg space, a number much larger than in the present species. The egg ribbon laid at the laboratory did not show further development. The egg ribbons collected from the field showed advanced stages of development.

Among the species of *Natica* pelagic development seems to be the normal feature. But in *Natica catena* both pelagic (Lebour, 1937) as well as non-pelagic (Thorson, 1946) development have been observed. Thorson (1935) observed purely non-pelagic development in three species of *Natica* from East Greenland and Knudsen (1950) in a single species from West Africa. Ankel (1930) observes nurse egg feeding in *N. catena*. In the present species, however, the development is pelagic and no nurse-egg feeding has been observed.

As soon as the larvæ are ready to hatch out the egg ribbon made up of agglutinated sand particles dissolves into a mass of loose sand grains. In the meanwhile the egg coverings surrounding the embryos give way, probably by dissolution, so that the larvæ can now lead a pelagic life. The veliger in the first few hours after hatching is very active. It measures 0·100–0·116 mm. across the shell. It is pale yellowish in colour. The velum is colourless and bilobed. The larval shell presents an uneven and rough surface. The foot and operculum are present, the former colourless and ciliated. The otocysts are fairly clearly seen as also the retractor. The eyes are black but the tentacles are not clearly visible. The alimentary tract as well as the liver are pale yellow.

The newly hatched veliger of this species could not be compared to that of the West African species because no details of veliger are given in the latter form except that the larva has a pelagic stage and the larval shell measures 100 μ across the shell. The veligers thrived at the laboratory for 4–5 days during which time they did not grow or metamorphose.

Natica tigrina (Röding) (Photo 10 and Figs. 31–35).—There is no previous record of the breeding habits of this species.

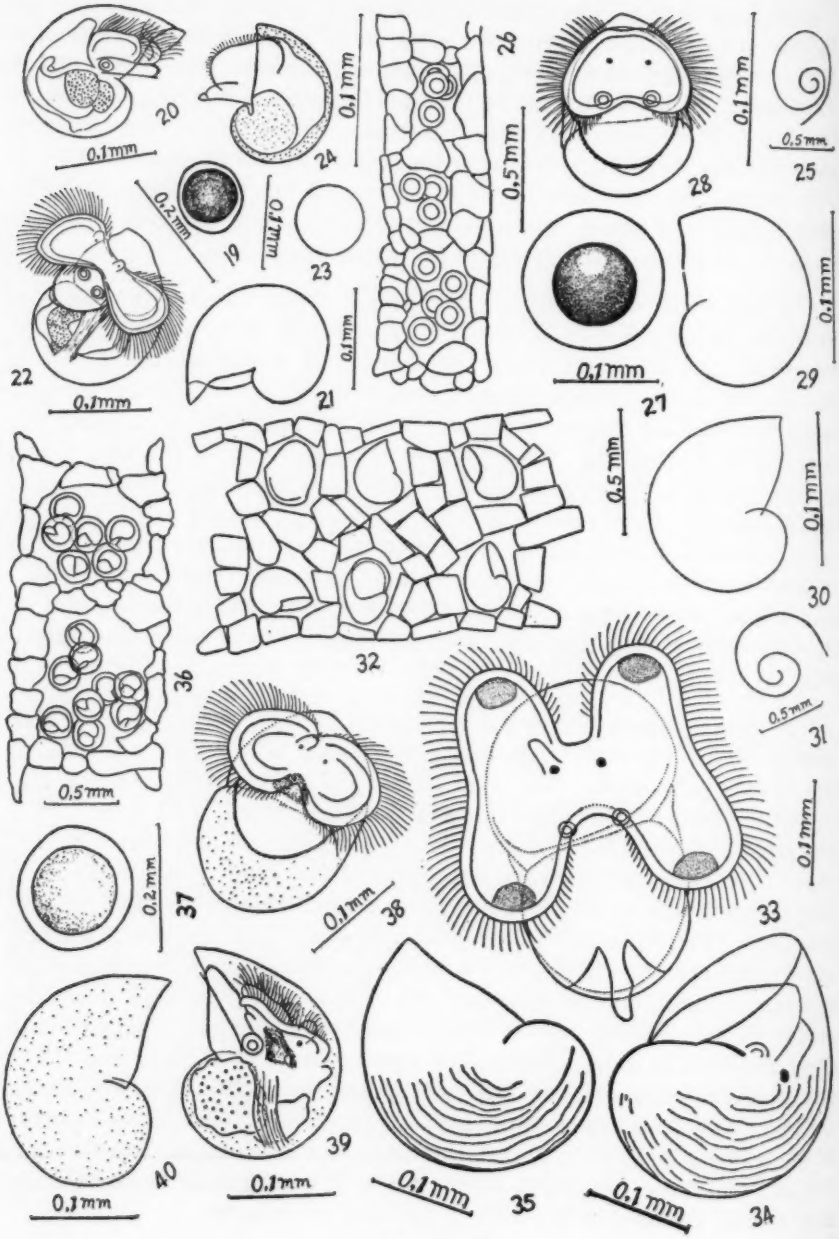
Egg ribbons bigger in size on an average than those of *N. marochiensis* were also met with in Kundugal Point area. No other adult specimens except *N. marochiensis* could be observed along with them. However in one of the collection trips two live specimens of *N. tigrina* and one *Polynices mamilla* were observed in the same area. The apices of the shells of these two species were examined. It is found that the larval shell of the veliger hatched out from the egg ribbon agreed with the apex of *N. tigrina* in the measurement of the 1st whorl. Hence it is possible that these egg ribbons belong to *N. tigrina*.

The egg ribbons were observed in the field in the month of January, February, March, May, July, August, September and December. It is likely that the breeding in this species takes place all the year round as in *N. marochiensis*.

The egg ribbons are in the form of an open ring but sometimes they are thrown into a spiral of 1½ rounds. They are greyish in colour with blackish tinge. A few egg ribbons, taken at random, measure 5·6–7·9 cm. across. They are about 165–192 mm. long (measured along the periphery), 23–28 mm. broad and 0·80–1·10 mm. thick. The inner margin of the egg ribbon is shaped into a ' collar ' which is more pronounced than in the previous species. This part of the egg ribbon is smooth and even but the periphery may be smooth or thrown into a series of characteristic folds. The spawn masses of *C. fluviatilis* are quite commonly found attached to the egg ribbons.

A transverse section of the egg ribbon shows about 110 egg spaces arranged distinctly in two planes (*i.e.*, about 55 egg spaces per plane) which are placed one above the other. Section of the ribbon lengthwise reveals that there are on an average 500 such rows arranged in two planes, each plane containing half the number. Roughly there are about 40–55 thousand egg spaces in an egg ribbon. The egg space measures 0·251–0·284 mm. across. Each egg space contains only one embryo unlike the previous species. Each egg or embryo is surrounded by an egg covering (capsule) which is transparent, colourless and flexible. Some of the egg ribbons revealed in the section the larvæ without the egg coverings. The latter were probably dissolved and the larvæ are now ready to lead a pelagic life. This is probably the stage when the egg ribbon disintegrates for the capsule-free larvæ to come out.

A ribbon collected on 8th August 1952 liberated the veligers on 13th August 1952. The embryo in the hatching stage shows thick and pinkish brown embryonic shell. The latter has very characteristic wavy spiral ribs as that of ' *Natica* sp. A.' from Iran figured and described by Thorson (1940). The embryo shows bilobed velum. Eyes, otocysts, foot and operculum are seen. The newly hatched veliger measures 0·200–0·250 mm. across the shell. The velum is well developed and has olive green pigment patches, two on each lobe. There is a sub-velum with minute cilia. The foot, operculum and otocysts are distinct. The foot is colourless and possesses a long contractile tentacle projecting at the distal end which is fully outstretched when the veliger swims. This feature is very identical with those of *Natica canrena* from Bermuda (Lebour, 1945). The operculum is white, thin and transparent. The eyes are present but only the right tentacle is developed at the time of hatching and the left one is not yet developed. The internal organs of the veliger are not distinctly seen through the sufficiently thick shell except for the heart-beat. The larvæ at this stage of development are commonly seen in the plankton samples from the Gulf of Mannar.


0.1mm
0.2mm
0.5mm
0.1mm
0.5mm
0.1mm
0.2mm
0.1mm
0.5mm
0.1mm
0.5mm
0.1mm
0.1mm
0.1

Natica sp. A. (Photo 11 and Figs. 36–40).—Egg masses belonging to *Natica* were collected from Shingle Island on 22nd February 1956. They were found on sandy areas. But no adults of *Natica* could be observed in the area wherein the egg masses were collected.

The greyish egg ribbon is encrusted with sand particles and is thrown into 1–1½ spiral. The length in a few egg ribbons examined is about 39·5 cm. along the outer margin and 18·2 cm. along the inner margin. The ribbon is 3·8–4·5 cm. in width and 0·84–1·29 mm. in thickness. The egg spaces are arranged in one plane. The largest diameter of the egg space is 0·50–0·84 mm. Each egg space contains a few eggs. The egg (preserved) measures 0·116 mm. It is surrounded by a thin-walled, flexible and transparent capsule which measures 0·167–0·217 mm. across.

The egg ribbon collected on 22nd February 1956 liberated the veligers 7 days hence. The newly hatched veliger measures 0·200 mm. across. The larval shell which shows one whorl is slightly brownish and has dotted surface. The velum is colourless and bilobed. Eyes are black. Only right tentacle is seen. It shows a few hairs at the tip. Foot is ciliated. Operculum and otocysts are present. The region around the mouth is purplish. Liver is yellow. The animal is yellowish. The larvæ thrived at the laboratory for two days but showed no changes during the time.

Natica sp. B. (Figs. 41 & 42).—Two egg masses having the features of *Natica* type were dredged near Kundugal Point in the month of January. The egg masses liberated the veligers even before reaching the laboratory. Therefore no details of the egg mass could be given except that the ribbon is fairly of large size and is light black in colour.

The newly hatched veliger measures about 0·634–0·651 mm. across the shell which has one whorl and begins to show some spiral striations. The embryonic shell is unsculptured. The velum is colourless and has a tendency to four lobes. It measures 0·75 mm. across in the middle. Each lobe measures about 1·10 mm. lengthwise. Sub-velum is present. Eyes, tentacles and otocysts are present. Foot is yellowish and well developed. The alimentary tract, particularly stomach, is purplish. The veligers did not thrive at the laboratory for more than 2 days.

Cypræidæ

The egg masses of *Cypræa* were described by several authors outside India (*cf.* Lo Bianco, 1899; Vayssiere, 1923, 1927; Ostergaard, 1950). Ray (1951) gives an account of the systematics, habits, habitats, etc., of cowries. But their breeding habits have not been described from India.

Erronea errones (Linné) (Photos 12 & 13 and Figs. 43–49).—An account of the breeding habits of this species was given by the present author (Natarajan, 1954). More details are given here. This species is quite common in the Palk Bay waters living in the reef and rocky areas between Munakkad and Mandapam. They appear to be limited to shallow waters as is usual with cowries.

The cowries were found perching over the egg masses which are laid on the undersurface or crevices of stones and boulders. The egg mass is thus hidden from view but the rather bulged foot and the completely immobile state of the animal suggest very clearly that the animal is brood-caring. The immobile animal appears to be in a state of tension as is seen from the expanded foot covering the egg mass, the extended mantle lobes covering the entire shell and the protruding siphon; when the animal is removed it becomes very uneasy and moves about the glass trough restlessly.

The breeding of this species was observed in the month of September (temperature 27·8° C., salinity 37·71‰), October (temperature 28·0° C., salinity 36·76‰), November (temperature 28·0° C., salinity 36·58‰), December (temperature 26·0° C., salinity 27·59‰), January (temperature 25·4° C., salinity 27·09‰), February (temperature 25·0° C., salinity 27·45‰) and March (temperature 24·4° C., salinity 28·33‰).

The egg mass is nearly circular about 23–26 mm. across, and 8–10 mm. in thickness at the centre. In the fresh condition it is yellowish in colour. It turns gradually pinkish brown and ultimately dull purple with the development of the larvæ. Five egg masses examined contained 481, 501, 507, 515 and 546 capsules respectively. The number of eggs per capsule ranges 20–76. All of them develop into embryos. It was observed that the capsules in the present egg mass are deposited in 5–6 layers superimposed upon one another, the diameter of the layer being largest at the base and showing gradual decrease towards the top. The pale yellow capsules are transparent and show variations in size and shape. They measure each 1·45–3·25 mm. long and 1·40–1·80 mm. broad. No preformed exit passage is present. The capsule is usually broad at the top and narrows down towards one side. It extends into a basal plate for attachment. The largest diameter of the base ranges 0·50–0.85 mm. In sections of the egg mass the mode of attachment of the capsule is clearly seen. The first layer is attached to the substratum. The capsules of the second layer attach themselves by their bases to the distal end or near about the distal area of the capsules of the first layer. The same arrangement is repeated by the third layer on the second and so on. The capsules are also glued sideways which give a compactness to the egg mass.

The eggs are yellowish (or yellowish-brown) in colour, and measure each about 0.263–0.267 mm. The newly hatched veliger measures 0.373 mm. across the shell. The latter is pale brownish in colour with reticulate markings on it. The velum is bilobed and colourless. There is a distinct subvelum with short cilia. The eyes are distinct but only the right tentacle is present and the left one is not yet developed. The statocysts are present. The foot is well developed, ciliated and shows diffuse black colouration. The operculum is colourless. While swimming the larva is either partly or fully surrounded by the mantle. The mouth, œsophagus, stomach and intestine are dark purplish in colour and are distinctly seen through the shell. The heart-beat is clearly visible. The liver is yellowish and appears pressed against the stomach. The larvæ thrived at the laboratory for about a week but no changes could be noted. The larvæ at this stage of development are very often seen in plankton samples from Palk Bay.

Tonnidæ (*Doliidæ*)

The egg masses of various species of *Tonna* (*Dolium*) were described in the literature (Thorson, 1940; Knudsen, 1950; Ostergaard, 1950). Panikkar and Tampi (1949) described a doliid egg mass from Krusadai area.

Tonna dolium (Linné) (Photos 14 & 15 and Figs. 50–54).—Egg masses belonging to *Tonna* were collected from Vedalai, Mandapam, Krusadai and Shingle Islands. Live specimens and shells of *Tonna dolium* have been observed in Shingle Island. The larval shell of veliger hatched out from the egg ribbon agreed with the apex of an young one of *T. dolium* in the measurements of 1st whorl. Thus it is likely that the egg ribbon belongs to the same species. This also confirms the identification of Dr. Thorson (*l.c.*) of a similar ribbon in Iran referred with doubt to *Dolium maculatum* (*T. dolium*).

The egg ribbons were observed in February, April and October. Usually the ribbons were found in broken pieces. This is particularly so in ribbons at advanced stages of development. Two of the ribbons (one of them bigger than the other) however are more or less complete. The dimensions as well as other features of the bigger ribbon are: length of the ribbon 36 cm.; breadth 8·4–9·5 cm.; thickness 1·71–1·87 mm.; number of egg spaces per row 38–41 (at the ends 20–25); total number of rows 125; largest diameter of the egg space 3·03 mm.; largest diameter of the exit hole 0·72 mm.; number of eggs per egg space 9²–101. The details of the smaller ribbon are: length of the ribbon 22·5 cm.; breadth 4·1–5·7 cm. (at the end 2·9–3·7 cm.); thickness 1·21 mm.; number of egg spaces per row 31–35 (at the end 11–26); total number of rows 123; largest diameter of the egg space 2·09 mm.; largest diameter of the exit hole 0·880 mm. Number of

eggs per egg space 29–32. The smaller ribbon is probably laid by an young one.

The egg mass is in the form of a gelatinous band. It is brownish in colour. The egg spaces are arranged in curved rows, and have each a flat bottom and convex top. They may have four or five sides or be circular or oval in outline. The exit hole may be elliptical or circular. The capsule itself is colourless and transparent.

The egg is spherical and brownish and measures 0·217-0·234 mm. across. The eggs are arranged in a semi-lunar mass in freshly laid egg ribbons. They are surrounded by white nutritive substance which the embryos utilise during the development. Roughly it takes about 15–17 days from egg to the time of hatching.

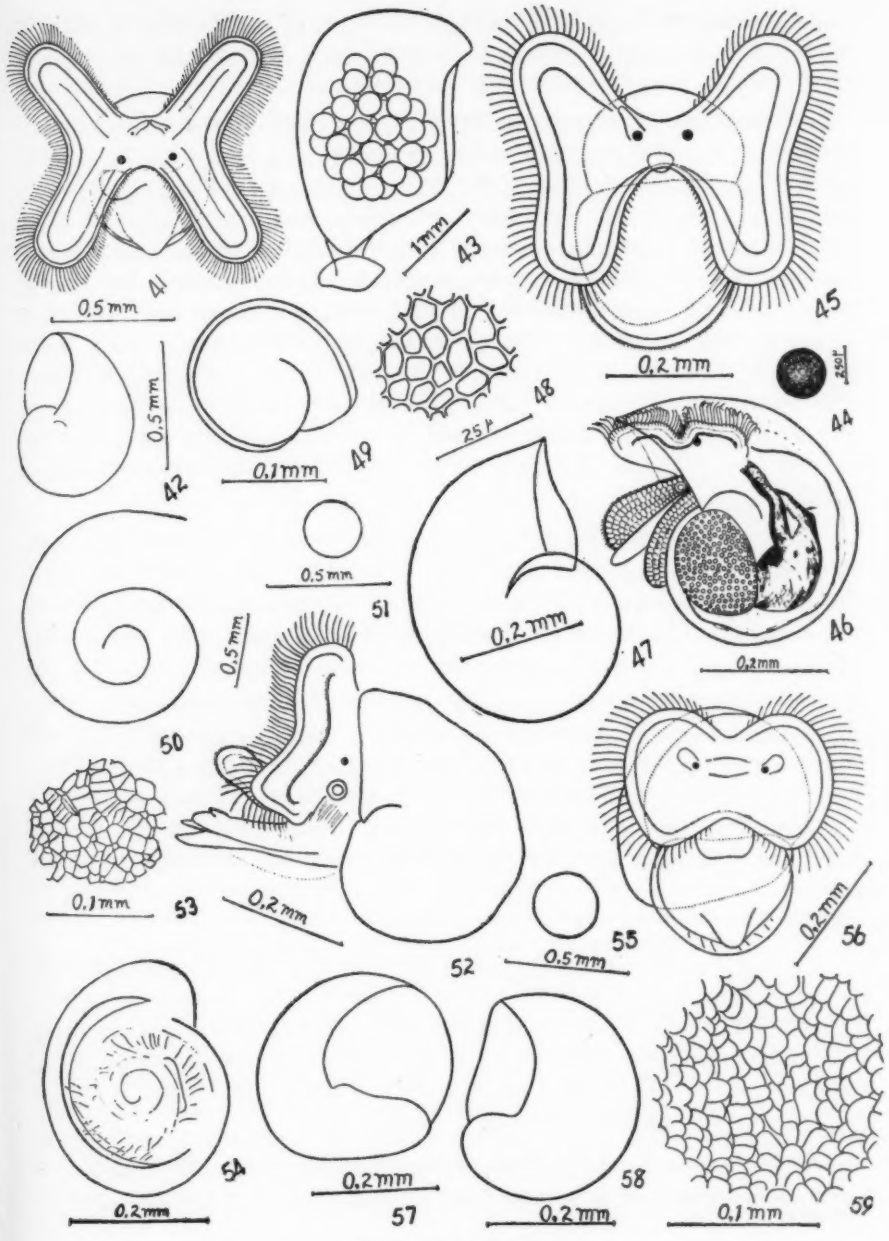
The newly hatched veliger measures 0·418 mm. across the shell which has one whorl and is brownish. The surface of the whorl is uneven and shows reticulate sculpture. The colourless velum is bilobed with a tendency to four lobes and measures 0·417 mm. across. Each velar lobe measures 0·367–0·468 mm. in length and 0·150–0·167 mm. in width. Sub-velum bordered with small cilia is present. Eyes are black. Only the right tentacle is seen with fine hairs at the tip. The foot has a ciliated tentacular process at the distal end. Ciliated proboscis is seen. The otocysts are distinct. The operculum appears to consist of two pieces, the outer one slightly jutting out. The liver is yellow.

The present observation is in agreement, in a general way, with that described for Iranian species by Thorson (*l.c.*). Besides normal development (*i.e.*, all the eggs develop into embryos), Thorson also observed in a few egg spaces each having a large embryo measuring about 2 mm. He was not quite sure whether this monstrous growth was due to real nurse egg feeding or 'cannibalism'. However such monstrous embryos were not found in the Indian species.

The doliid egg mass described by Panikkar and Tampi (*l.c.*) from Krusadai area also seems to belong to *T. dolium* only.

Tonna sp. (Photos 16–18 and Figs. 55–59).—Egg ribbons belonging to *Tonna* have been collected from Krusadai, Shingle Island, Vedalai and Mandapam. But they could not be referred to the adults with certainty.

The egg masses were observed in the month of January (temperature 24·5° C., salinity 27·23‰), May (temperature 30·5° C., salinity 33·75‰), July (temperature 20·0° C., salinity 33·69‰) and November (temperature 27·0° C., salinity 36·42‰).


0.5 mm
41
1mm
43
0.2 mm
45
0.5 mm
42
0.1mm
49
25 μ
48
250 μ
44
0.5 mm
51
0.2mm
47
0.2mm
46
0.5 mm
50
0.1 mm
53
0.2 mm
52
0.5 mm
55
0.2mm
56
0.2 mm
54
0.2mm
57
0.2 mm
58
0.1 mm
59

FIGS. 41–59

The egg mass is a gelatinous band or ribbon. It is brownish or pinkish yellow, but colourless in the spent up condition. They are usually washed ashore and never attached to any substratum.

The ribbons measure about 292 mm. long, 54–80 mm. broad and 1·6–1·9 mm. thick. The egg space (capsule) may be 4, 5 or 6 sided or circular or oval. Its largest diameter is 2–2·65 mm. The wall of the egg space is smooth and transparent and has on the convex face a preformed exit hole which is very vague or unpronounced in the freshly laid ribbon, but becomes clearer at the time of hatching of the larvæ. It is circular, elliptical or oblong in shape, having largest diameter of 0·88–1·00 mm. The exit hole is covered by a thin membrane which gives way at the time of hatching. About 31–38 (and 12–13 at the ends) egg spaces are arranged side by side in curved rows. There are about 117 such rows and 3,300 capsules in a ribbon. The number of eggs in an egg space ranges between 95 and 110. All the eggs seem to develop into embryos. Roughly about 336,000 embryos emerge out of a single ribbon.

The eggs are yellowish or brownish yellow and measure each 0·233 mm. across (based on preserved material). The freshly laid ribbons in which the eggs are grouped in a semi-lunar mass in each capsule, take about 17 days to hatch out. The eggs are surrounded by a white thick sheet of nutritive fluid.

The yellowish embryos emerge out as large veligers through the exit hole. They measure each 0·317–0·334 mm. across the shell, which shows about a whorl, is pale yellowish and presents a characteristic reticulate sculpture. The velum is colourless and has more or less two equal lobes. Eyes and tentacles are seen. Foot and operculum are present. The otocysts are present but quite small and indistinct. The veligers thrived at the laboratory for two days.

Muricidæ

The egg masses of some species of Muricidæ are known in the literature (Annandale and Kemp, 1916; Hornell, 1921; Lamy, 1928; Burkenroad, 1931; Risbec, 1932, 1935; Fischer and Raffy, 1933; Mohr, 1933; Lebour, 1937, 1945; Thorson, 1940; Gravely, 1942; Knudsen, 1950; Ostergaard, 1950; and Chari, 1950). The egg cases common at Tuticorin and referred to *Murex* by Hornell (1921) appear to belong to *Thais*.

Murex virgineus var. *ponderosa* Sowerby (Photo 19 and Figs. 60–69).—The breeding habits of this species are described here for the first time.

Satyamurti (1952) has recorded this species from Kundugal Point and Pamban. It has also been collected in large numbers at Seeniyappa Dharuga and Vedalai regions.

An adult specimen of this species measuring 6·5 cm. in length and 4·4 cm. in breadth laid an egg mass on 8th December 1954 on the glass of the aquarium close to the surface of the water, the temperature and salinity being 24·2° C. and 29·25‰ respectively. It took about 42½ hours to complete the spawning process which resulted 61 capsules being laid. The adult was transferred to a glass trough and removed to the laboratory for observation. The same specimen laid one more capsule on the bottom of the glass trough after 5½ hours; another specimen of a length of 7·3 cm. and largest breadth of 4·6 cm. laid an egg mass consisting of 94 capsules and took about 39½ hours; a third one of a length and breadth of 6·7 cm. and 3·9 cm. respectively laid 91 capsules in about 34½ hours. In the second and third cases the salinity and temperature were more or less the same as the first one.

The breeding in this species was observed in November, December, January, February, March, May, June and July. It is likely that the breeding in this species is all the year round.

The pale yellow capsules are broad at the top and become narrower towards the base. They are slightly flattened bounded by two strong longitudinal ridges, one on each side. On one face there is a groove in the middle which is broad and much pronounced at the top and becomes narrower and shallower towards the base; in some the groove is indistinct beyond the middle of the capsule. The other face is smooth. The length and the largest breadth of the capsule are 9·5–11 mm. and 4·6 mm. respectively. The capsule presents an elliptical outline in cross-section. Its top, viewed from above, is kidney-shaped and has an edge which curves upwards. In the centre there is a circular or elliptical exit hole which measures 1·3–1·4 mm. and is covered by a thin, transparent membrane. Each capsule has a well-developed basal plate and the bases are confluent so that there appears to be a broad sheet from which these capsules stand vertically.

The eggs which are white and measure each 0·200–0·217 mm. across, are suspended all over inside the capsule in a gelatinous, colourless, thick fluid. The number of eggs per capsule ranges 440–731. Of these only a few develop into large embryos while the rest form nurse eggs. The newly laid capsules show embryos in the hatching stage in about 20 days. At the time of hatching the number of embryos per capsule ranges 9-30.

The covering membrane of the exit hole gives way at the time of hatching. The embryos at the hatching stage show remarkable variations in size ranging

0·54–1·83 mm. But about 75% of them fall within 1·18–1·75 mm. range. It is not possible, at the moment, to attribute any specific causes but it is likely that this variation may be due to the disparity in the consumption of nurse eggs by the embroys, *i.e.*, there may be some sort of competition among the embryos in the consumption of nurse eggs.

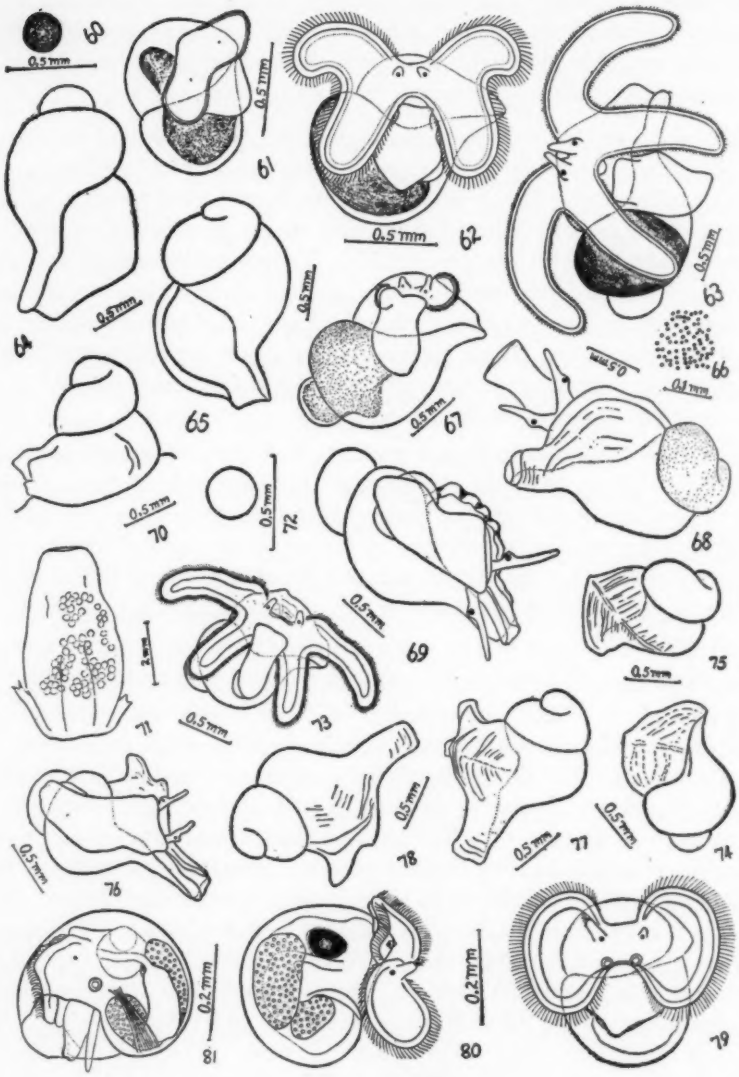
The embryo examined at an early stage has hardly one whorl. Velum is bilobed and is bordered with small cilia. Eyes are small and the tentacles not yet developed. Foot is present but the otocysts are not clear. The embryo itself is little differentiated and apparently only contained a yolky mass. Heart-beat is visible.

At the next stage the embryo has a shell of about two whorls the second whorl showing granulated surface. It has a shell-siphon. The velum shows a tendency to four lobes. The opercular structure of the foot is now seen. The tentacles are now developed. The embryo is still little differentiated. The embryo about to hatch out has a shell of 2½ whorls, showing a well-developed shell-siphon. The velum is distinctly four-lobed, each lobe being narrow and long. The larva partly crawls and partly swims, a feature denoting short pelagic period. The embryos did not hatch out in the laboratory condition. It is very likely that they emerge out of the capsule at this stage as free swimming veligers.

After 2 days the veliger (measuring about 1·78 mm. in the present case) begins to lose the velum. The velum is reduced into two lobes, the right lobe is smaller than the left and about to disappear. In about 2½ days the velum is completely lost and the young ones, in the present case, measure each about 1·85 mm. The foot is well developed and the young ones crawl about. The operculum is brownish. The eyes and tentacles are very well developed. The young ones thrived at the laboratory for about 20 days. They have now 2¾–3 whorls and measure each 2·05 mm. across the shell.

REMARKS

The nurse egg feeding, in this species, is worth mentioning. The embryos at early stages were dissected out from the egg capsules and kept in a watch-glass along with nurse eggs. They reach the nurse eggs and each egg is manipulated towards the mouth with the help of the velar lobes and the foot. Then they press the egg with the velar lobes. The egg collapses due to pressure. Then the eggs are gradually sucked up which do not appear to take more than 2 minutes. This process of nurse egg feeding appears different from those of *Nucella* and *Buccinum* types (Thorson, 1946).



FIGS. 60–81

Murex trapa Röding (Photo 20 and Figs. 70–78).—The adult specimens of this species have been collected in large numbers from Vedalai and Pudumadam areas. The present egg masses collected from Vedalai and Pudumadam in November and December 1955 liberated the veligers whose larval

shells begin to show the long anterior canal and processes thereby revealing their affinity to *Murex trapa*. The apex of an young one of *Murex trapa* also agreed broadly with the larval shell. The present egg masses are therefore referred with some certainty to this species.

The egg mass has a characteristic structure. It consists of a number of cone-like structures, more often appearing as though budding from a primary cone. Each cone-like structure is really formed of a number of small capsules which are strung in a regular pattern resulting in hollow cone with diamond-shaped cavities to the outside. These cavities communicate with the internal main or axial cavity of the cone. Individual capsules are united only externally by basal ends while the distal ends are free and compact opening to the axial space. There are as many as 24–36 cone-like protuberances in an egg mass. Each of them consists of 107–167 capsules. On an average there are 4,110 egg capsules in an egg mass.

The capsule is a compressed pouch. It is fairly transparent showing the embryos inside. The length and the largest breadth of the capsule are 5·0–7·5 mm. and 2·5–3·75 mm. respectively. The top of the capsule forms the exit hole for the emerging larvæ. It is circular or elliptical and is covered by a thin membrane. The largest diameter of the exit hole is 1·32–1·49 mm. The lacuna of the pouch is 0·94–1·76 mm. Each capsule has a base but no stalk or peduncle.

The number of eggs in a capsule ranges 163–204. They are suspended in a mucous-like fluid. They are spherical and yellowish and measure each 0·267 mm. It takes about 20 days from the time the eggs are laid to the time of hatching. In this species all the eggs do not develop into embryos. Only 10–26 embryos appear to develop, in each capsule the rest of the eggs subserving as nurse eggs. In this case, therefore, the embryos attain a large size before emerging out by feeding on nurse eggs and surrounding fluid. The embryos did not hatch out in the laboratory condition. However they were dissected out from the capsules and kept in finger bowls. The velum of the larva is four-lobed. Sub-velum is present with small cilia. The width of the velar lobe is about 0·334 mm. The eyes are black. The tentacles are fairly prominent. The foot is well developed with blackish tinge. The operculum is present. The otocysts are not clear. The liver is yellowish. The larval shell measures 1·00–1·27 mm. It has $2\frac{1}{3}$ whorls and is pale brown. The last whorl shows angulation in the middle as well as transpiral striations. The outer lip of shell is extended forward in the middle. The larva settled down in about $2\frac{1}{2}$ days losing velar lobes. The young one examined after two days shows the shell of 1·70 mm. in length. The anterior canal of the

shell is more elongate. The outer lip of the shell begins to show processes and transpiral ridges. The foot is well developed. The eyes are small. The tentacles are long and slender. The young one thrived for about 12 days at the laboratory. The 12-day old young one shows further growth and has a shell of 1·85 mm. in length. Anterior canal shows further prolongation.

Thais bufo (Lamarck) (Photos 21 & 22 and Figs. 79–81).—Egg clusters of *Thais* were collected from Vedalai and Mandapam. In these areas both *T. rudolphi* and *T. bufo* are well represented. A single young specimen of *T. rudolphi* showed the apex more or less intact. The apex was compared with the larval shell of the veliger hatched out from the egg cluster and was found larger in the measurement of 1st whorl. Therefore it is very likely that the egg cluster belongs to the other species namely *T. bufo*. Direct comparison of the apex of *T. bufo* with the larval shell was not possible as the young ones of *T. bufo* with apex intact could not be collected. A brief account of the egg capsules of *Thais bufo* has been given by Gravely (1942) from Madras and Chari (1950) from Mandapam.

The breeding of this species was observed in January–June period. The egg clusters are glued generally underneath the jutting edges of rocks or in their crevices. They usually comprise of yellow, violet and grey capsules. The yellow capsules showed embryos in their early development and grey ones, embryos in the hatching stage. The violet capsules show a column of yolk probably due to the disintegration of egg or embryo. The clusters are generally laid close to each other and it is therefore difficult to ascertain the number of egg capsules laid by a single female.

The egg capsules are like small cylindrical tubes with long stalks and confluent bases. The distal end is slightly bulged. The top of the capsule bears an exit hole which may be circular or elliptical and is covered by a transparent membrane. The exit passage measures 0·55–0·60 mm. across. The length (excluding the stalk) and largest breadth of the capsule are 5·00–7·00 mm. and 1·50–2·05 mm. respectively. The stalk is slender, long and flattened and is 3–5·5 mm. long. Its largest and smallest breadth are 0·450–0·728 mm. and 0·250–0·450 mm. respectively. The number of embryos per capsule is 254–353.

The egg capsules contain a colourless fluid. They are almost packed with embryos at the time of hatching. The thin membrane covering the exit hole gives way at the time of hatching. It is interesting in this connection to note that the larvæ of *Thais hippocastaneum* never use the exit hole but come out through a hole in the side wall which they have rasped out themselves (Thorson, 1940).

Among purpurids the development may be pelagic or non-pelagic (Lebour, 1937; Thorson, *l.c.*). *Thais hæmostoma* provides a fine example of pelagic as well as non-pelagic development in the same species in different areas. It is pelagic in Lousiana (*cf.* Burkenroad, 1931) and non-pelagic in West Indies (Korschelt and Heider, 1936). Nurse egg feeding has been noted in *T. hippocastaneum*. In the present species the development is pelagic and no nurse egg feeding has been observed.

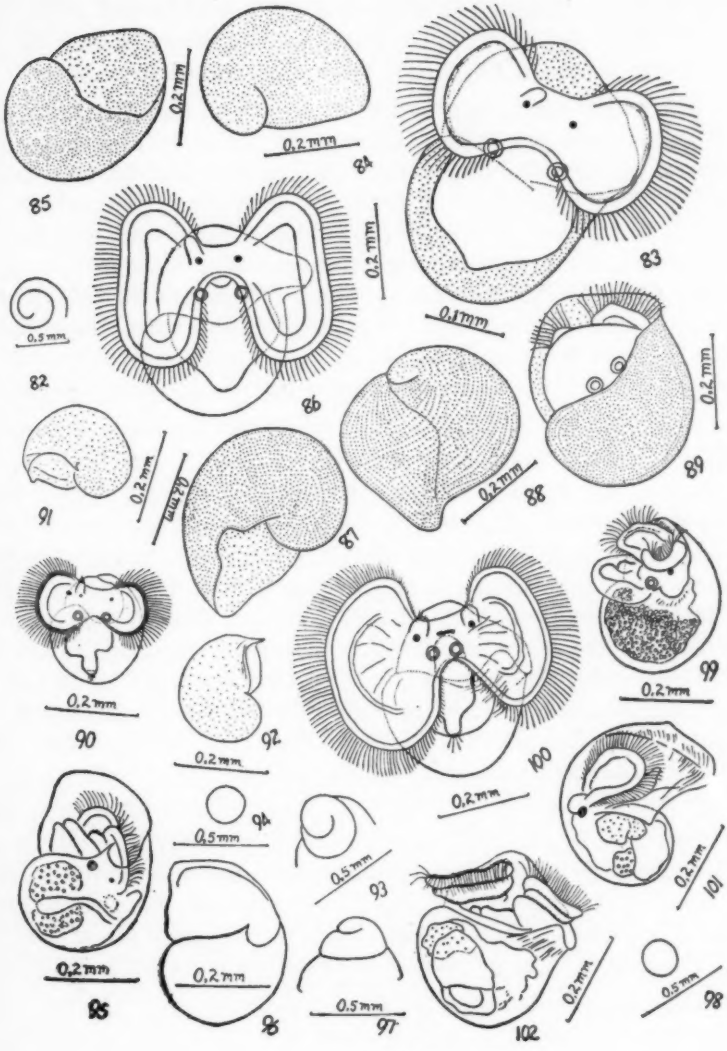
Embryos examined at the time of hatching measure each 0·283–0·367 mm. across the shell. Of these, larvæ of 0·334–0·367 mm. size seem to be predominant. In certain capsules some of the embryos are far less developed measuring only 0·217 mm. These embryos appear to form only a small negligible percentage.

The newly hatched veliger (measuring 0·334–0·367 mm.) has a shell of just more than one whorl. The shell has beaten surface as is seen in *T. carinifera*. The velum is bilobed and colourless. There is a distinct sub-velum with small cilia. The eyes are black and distinct. Only the right tentacle appears well developed and the left one is just beginning to show up. This disparity of development in tentacles in newly hatched larvæ have been met with in many other species. There are few long hairs at the tip of right tentacle. The foot and operculum are present, the former is ciliated. The otocysts are distinct. The liver is yellow. The alimentary tract is of purple colour especially the stomach and the anal region. There is a dark purplish pigment spot in the region of anus. The veliger thrived at the laboratory for 2½ days, without showing any further change.

Thais tissoti (Petit) (Photos 23 & 24 and Figs. 82–85).—There is no previous record of the reproductive habits of this species.

Six egg masses belonging to purpurid type were dredged on 13th December 1954 near Rameswaram Road at 4 fathoms. *Thais tissoti* and *Drupa heptagonalis* were the only forms that were collected in that area. The larval shell of the veliger, liberated from the egg mass, agreed broadly with the apex of *Thais tissoti* in the measurement of the 1st whorl. It is therefore likely that the egg masses belong to the same species. Satyamurti (1952) has recorded this species at Pamban.

The egg masses were attached to the brown algæ *Spathoglossum asperum*. They consist of 80, 55, 59, 25, 75 and 62 capsules respectively. The largest diameter of the egg mass is 6–11 mm. The capsules are laid at a distance of about 0·50 mm. from each other. The cylindrical capsule is pale brown and has a smooth, colourless, transparent surface. It has no stalk unlike the preceding species and the well-developed basal plate of each capsule



FIGS. 82–102

merge completely with each other to form a common base. The distal end of the capsule has a smaller diameter (0·45–0·50 mm.) than the rest of the capsule and has a flattened top which is provided with a thin membrane. Here there is no preformed exit hole but the entire top forms the exit passage

for the emerging larvæ. The length and the largest breadth of the capsule are 1·90–2·50 mm. and 0·70–1·90 mm. respectively. The number of the embryos per capsule is 19–32. All the eggs develop into embryos.

The embryos about to hatch out occupy almost the entire capsule. The larval shell is thin and fragile and measures about 0·317 mm. across. Its surface shows beaten appearance. The edge of the aperture is slightly brownish. The shell-siphon is beginning to appear. The animal is pale yellow. The velum is bilobed and shows brownish colouration on the border of velar lobes. Sub-velum is present but not distinct enough. The eyes are distinct. Only the right tentacle is present. The foot is ciliated. The operculum and otocysts are present. A violet spot is present near the area of anus. The liver is pale yellow.

The newly hatched veliger thrived at the laboratory for 2 days. The larval shell now measures 0·350 mm. The rather small sized velum of the veliger point to short pelagic period.

Thais sp. A. (Photo 25 and Figs. 86–88).—No previous account is available of the egg mass of this species. Egg clusters attached to the blades of the seaweed *Gracilaria corticata* (Rhodophyceæ) were found entangled in the shore-seine operated in the Pudumadam areas. The smooth, unridged capsules are laid close to each other at a distance of 0·55–0·75 mm. They are cylindrical and fairly transparent. The top of the capsule which forms the exit passage for the emerging larvæ is flat or slightly convex and provided with a thin membrane. The largest diameter of the exit passage is about 0·65 mm. The basal plates of the capsules are confluent and forms a broad sheet. The unstalked capsule is slightly bent or curved. It is not uniform in width and is largest in the middle. The length and the largest breadth of the capsule are 3·75–5·50 mm. and 0·90–1·15 mm. respectively.

The newly laid egg clusters are quite yellowish due to the yellowish eggs. The eggs which were in the early stages of development measured 0·200 mm. across. They were evenly distributed inside the capsule. All of them develop into embryos. The number of the embryos per capsule is 57–92.

It takes roughly 4–5 days for the egg to hatch out. The larvæ about to hatch out could be seen moving about actively inside the capsule. The larval shell measures 0·367 mm. across and has beaten surface. The shell-siphon is slightly brownish. The larvæ are yellowish. The velum is bilobed. Sub-velum is present. Eyes are distinct. Only the right tentacle is present. It has few fine hairs at the tip. The foot and operculum are present, the former is ciliated and the latter is whitish in colour. The otocysts are prominent. The stomach and anal part of the alimentary tract are pale-pinkish.

The tissue near the anal area is purple and the liver is yellow. Heart-beat is visible. The newly hatched veligers thrived at the laboratory for about three days.

Thais sp. B. (Photo 26 and Fig. 89).—The egg mass of this species was not described before. The egg clusters, attached to shells or seaweeds, were collected from Pudumadam in the month of December 1954. They are thick yellowish when freshly laid and fade into dull grey with the development of the larvæ. The capsule has no stalk and is bent on one side. It has a more or less uniform width excepting the apical area where it extends into a nipple-like structure. The capsule is pillar-shaped and recalls that of *Thais carinifera* figured and described by Thorson (1940) from Iran. The nipple is thin and transparent and breaks off at the base at the time of hatching and leaves an exit passage for the outgoing larvæ. The capsule is smooth and semi-transparent. The length and the largest breadth of the capsule are 8–10 mm. and 1·15–1·30 mm. respectively. The average number of eggs per capsule is 146.

The eggs are yellow and are already in the early stages of development and measure each 0·200–0·250 mm. across. It may take about 4 days for the eggs to hatch out as free swimming veligers. The newly hatched veliger measures 0·417 mm. across the shell which shows the beaten appearance. Unfortunately the larvæ were preserved by mistake before making a detailed examination in the living condition. However, the following details may be given: The velum is colourless and bilobed. It is bordered with long cilia. The eyes, foot, operculum and otocysts are distinctly seen.

Thais sp. C. (Photo 27 and Figs. 90–92).—The egg masses collected from Shingle Island in the month of February 1955 have all the features of the egg cluster of *Thais* but could not be referred to the species with certainty. They were found attached to the crevices of the rocks and stones.

The egg clusters consist each of about 60–80 capsules. The length and the largest breadth of the capsule are 3–4 mm. and 1·59–1·82 mm. respectively. The capsule is vase-shaped and opens at the distal end by a short neck covered by a thin membrane which gives way at the time of hatching. Viewed from above, the exit passage shows a more or less elliptical outline which has the largest diameter of about 0·935 mm. There are about 354–392 embryos in each capsule.

The newly hatched veliger measures 0·183–0·233 mm. across the shell. But the larvæ measuring 0·200–0·217 mm. appear to be predominant. The shell is brownish and shows one whorl. It has beaten surface. The velum

is bilobed and shows brownish pigmentation along the margin. Sub-velum is present. It also shows a line of brownish pigmentation. Eyes are black. Only right tentacle is seen. It shows a few fine hairs at the tip. Foot has a characteristic shape and is ciliated. Besides a few fine hairs, there are a few dark pigment dots at the free end of the foot. Otocysts and operculum are present. Liver is yellow. Retractor is seen. The animal is yellow. The veliger thrived at the laboratory for 2 days.

Pyrenidæ (*Columbellidæ*)

The egg capsules of various species of *Pyrene* (*Columbella*) have been described by previous workers (Petit and Risbec, 1929; Thorson, 1935, 1940; Franc, 1943; Knudsen, 1950; Amio, 1955). No details seem to be available regarding the breeding habits of any species of *Pyrene* from India.

Pyrene flavida (Lamarck) (Photos 28 and 29).—Live specimens of this species were observed in Pamban sticking to dead corals on the reefs. Adult specimens kept at the aquarium towards the end of August 1954 laid an egg mass on 20th September, the egg mass being attached to the glass of the aquarium very near the surface of the water. Temperature and salinity at the time of spawning were 27° C. and 35·01‰ respectively.

The egg mass as a whole has a length of about 2·1 cm. and a largest width of 1·2 cm. It consists of 30 egg capsules. Each capsule has a very conspicuous basal disc with which it is attached to the substratum. The basal plates of all capsules tend to fuse or merge with each other.

The capsule looks helmet-shaped with its adhesion disc. It is a little pressed in the middle on one side. On the opposite side is seen a keel in some capsules and in others it is not clear or absent. There is no peduncle. The basal plate is round or longish oval. The largest diameter of the adhesion disc is 3–4 mm. The length and breadth of the capsule are 1·70–3·15 mm. and 2·15–2·75 mm. respectively. The number of eggs per capsule is 3–24.

The capsule which is pale brownish in colour is unsculptured and is fairly transparent so that eggs are visible through the wall. The wall of the capsule at the top is very thin and probably forms the exit passage for the larvæ at the time of hatching. Petit and Risbec (*l.c.*) described the egg capsules of the same species (*C. flava*) from New Caledonia. The New Caledonian species has more than semi-globular egg capsules with a keel at one side. It has no peduncle and is attached to the substratum by a basal disc. The columbellid egg capsules are of different types (Thorson, 1940). Thus in *C. tringa* the capsule is cucurbiform and is attached to the substratum by a

short stalk. In *C. versicolor* the capsule is more than semi-globular with one or two keels without stalk and possess a flattened area at the top of the capsule separated from the side walls by a faint keel. In *C. blanda* this keel has developed into a collar-like membrane and in *C. rosacea* two collar-like membranes are present on the outer surface of the capsule. In *Pyrene misera* (Amio, 1955) the capsule is dome-shaped with an adhesion disc. At the top there is an elliptical origin of exit hole between two indistinct keels.

Nurse egg feeding seems to be the common phenomenon in Columbellidæ and this may be related to the tidal zone habitat of most of the members of this family. This is borne out by the example of *C. blanda* (Thorson, 1940) and *C. rustica* (Franc, 1943). Petit and Risbec find about 20 eggs per capsule in *C. flava* of New Caledonia. Of these eggs only half the number undergo regular segmentation and the rest are unfertilised ones. In the present species there is an average number of 13 eggs per capsule. The egg is yellowish and measures 0·250 mm. across. They appear to be suspended in a thick colourless nutritive fluid. Unfortunately the egg capsules were attacked by swarms of ciliates and further observation was made impossible. A few capsules examined showed that some of the eggs did not undergo segmentation at all and a few were in a state of disintegration. Further investigation is necessary to ascertain whether there are nurse eggs in this species.

Pyrene versicolor (Sowerby) (Photo 30 and Figs. 93–96).—The egg capsules belonging to *Pyrene* were collected from Manoli Island in the month of February 1955. They were attached to seaweeds. *Pyrene versicolor* appears to be quite common in the area examined and the egg capsules are therefore referred to this species. The apex of the adult shell also shows correspondence with the larval shell of the newly hatched veliger in the measurement of the 1st whorl, thereby adding weight to the identification.

The capsule is more or less circular or oval in outline. It is plano-convex and is white and transparent. The convex surface of the capsule shows a number of concentric ridges interlaced by disconnected radial ridges resulting in distinct pattern of rectangular spaces towards the periphery and a more or less reticulate netted appearance towards the top which appears to be due to the irregular folds of the wall of the capsule. The exit hole placed in the centre of the top of the capsule is elliptical and covered by a membrane which has highly wrinkled surface and appears superficially reticulate. The rim of the capsule is thickened and distinct. The basal plate or membrane appears to extend as a broad edge to the capsule. The capsule measures (excluding the edge) 1·80–2·00 mm. The edge has a width of 0·22 mm. The largest diameter of the exit hole is 0·609–0·696 mm.

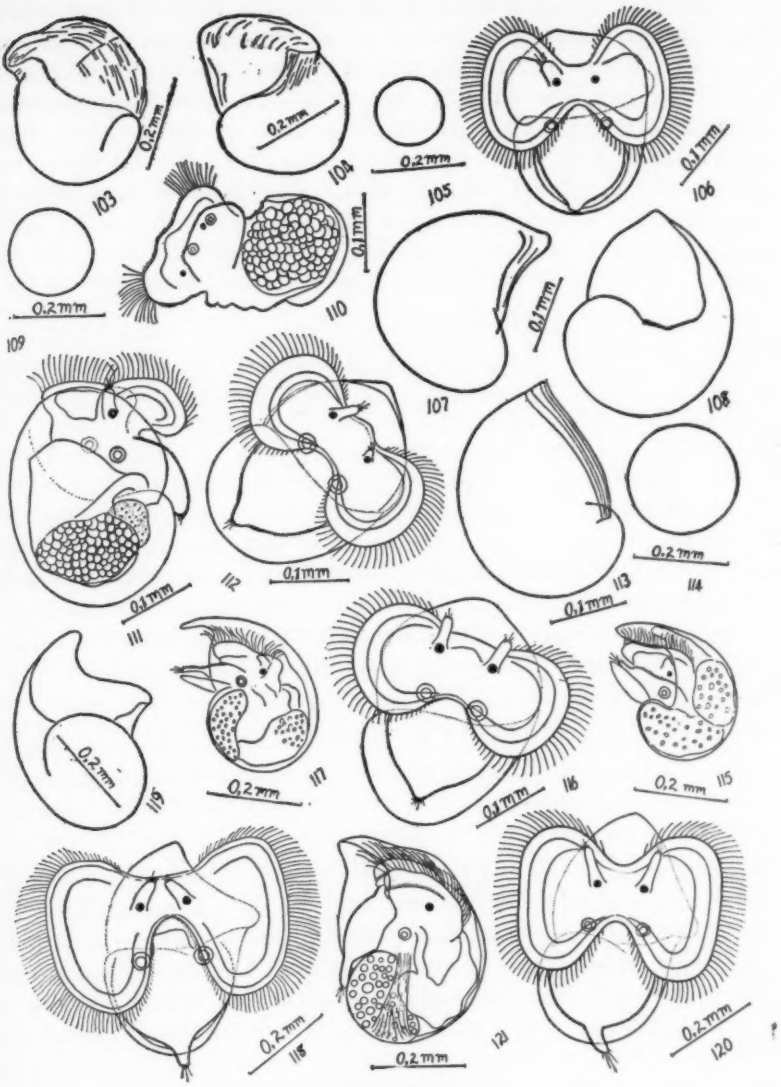
The eggs are white and spherical and measure each 0·184 mm. across. They are suspended in a colourless fluid. There are about 40–50 eggs in a capsule. All of them appear to develop into embryos. The eggs do not show normal development in the laboratory condition. Capsules containing embryos in the hatching stage however liberated the veligers. The newly hatched veliger measures 0·317 mm. across the shell. The shell is fairly transparent and has one whorl. The velum is bilobed and colourless. Right lobe appears bigger. Sub-velum is present. Eyes are black. Tentacles are seen. Foot and operculum are present, the former is ciliated. Liver is yellow. Alimentary tract is purplish. Heart-beat is visible. The animal is yellow. The veliger thrived at the laboratory for 2 days.

The egg capsules attributed to *Pyrene versicolor* by Risbec (1929) and Amio (1955) seem to have different features from the present capsule. Possibly they belong to different species of *Pyrene*.

Pyrene zebra (Gray) (Photo 31 and Figs. 97–104).—The breeding habits of this species are described here for the first time.

The adult specimens of this species appear to be quite common in the gulf of Mannar in the areas between Vedalai and Mandapam. The present egg capsules collected from the same area seem to belong to *Pyrene*. The newly hatched veligers from the egg capsules show larval shells which agree with the apex of *P. zebra* in the measurement of the first whorl. Hence the present egg capsules are referred to the same species. The present egg capsules seem to belong to the type of *P. misera*.

The egg capsules may be laid isolatedly or attached to one another on the sides into a compact structure. They are oval or circular in outline and are plano-convex. Each has a distinct edge which appears to be the extension of the bottom membrane or plate. The surface of the capsule has a wrinkled appearance and shows vaguely in some of the freshly laid capsules 2–4 broken concentric lines towards the top. At the top of the capsule is present an elliptical exit hole which is covered by an opaque membrane. The membrane shows reticulated sculputre. Six of the capsules taken at random measure (excluding the edge of the capsule) 1·93×1·76 mm., 1·93×1·71 mm., 1·71×1·60 mm., 1·93×1·82 mm., 2·20×1·88 mm. and 2·15×1·98 mm. respectively. The edge of the capsule is 0·28–0·33 mm. The exit holes in four capsules, taken at random, measure 0·77×0·55 mm., 0·66×0·50 mm., 0·66×0·50 mm. and 0·77×0·66 mm. respectively. The capsule seems to be attached to the substratum by the edge only. It is white and transparent showing clearly the developing embryos inside.



FIGS. 103–121

The egg is white and spherical and measures 0·184 mm. across. The number of eggs found in seven capsules are 22, 37, 36, 32, 29, 27 and 37 respectively. They appear to be suspended in clear colourless fluid.

The embryo examined before hatching measures 0·283 mm. across the shell which is thin and transparent and shows one whorl. The shell-siphon shows sculpture in the form of lines. The velum is bilobed. The right lobe is slightly bigger than the left. The velar lobes show bluish pigmentation along the margin. This pigmentation is more or less indistinct and only seen in closed light. There is a sub-velum with short cilia. Eyes are black and distinct. Only right tentacle is seen. The foot has a characteristic shape and is ciliated. It also shows a few long hairs at the distal end. The operculum is quite large and white. The otocysts are distinct. The internal organisation of the larvæ is not yet differentiated. Heart-beat is visible.

In the day-old veliger the disparity in the size of the lobes is quite marked. Eyes are prominent. Both tentacles are present. They also show a few hairs at the tip. In the area between the tentacles there is a patch of bluish pigmentation. The foot also shows on the left basal margin a patch of bluish pigmentation. The shell now measures 0·334 mm. The outer-lip of the shell is slightly inflexed in the middle. The shell also shows a distinct spiral line besides transpiral striations. The internal organisation of the larvæ is now differentiated. The liver is golden yellow. The alimentary tract shows a patch of bluish pigmentation immediately after the stomach which also shows a few bluish dots. This feature is clear in some of the larvæ, vague and indistinct or absent in others. The 2-day veliger shows further growth and now measures 0·350 mm. The well-developed velum suggests that the larva may have a long pelagic life.

Pyrene sp. (Photo 32 and Figs. 105–108).—The egg capsules were found usually attached to the surface of the egg capsules of *Murex virgineus* var. *ponderosa* and *Conus*. They could not be referred to the species with certainty.

The capsules are usually laid close to each other. The edge of one capsule usually touches or overlaps the next. But in many cases capsules are quite apart from one another. The dome-shaped capsule is surrounded by a well-developed edge. It appears to be fixed to the substratum mainly by the edge as is evident from the fact that when the edge is detached from the substratum the entire capsule comes off without difficulty. The capsule is white or colourless, thin and transparent revealing the eggs inside. It is not smooth but shows a wrinkled surface as though it is shrunk. It may be round, or oval in shape. In or around the centre of the capsule there is a preformed exit hole of a diameter of about 0·35 mm. It is round or elliptical and is covered by a thin membrane. The largest diameter of the capsule (including the edge) is 2·05–3·05 mm. Two faint ridges run in opposite

directions from the centre of the capsule towards the periphery. In some the ridges are vague. In the following table the number of eggs in relation to the largest diameter of the capsule is given for a few capsules.

TABLE I

Largest diameter of the capsule (excluding the edge) (mm.)	2·25	2·00	2·30	1·80	2·50	2·05
Number of eggs per capsule	88	77	90	61	103	82

The eggs are spherical, yellowish or brownish yellow and are found all over inside the capsule. They are surrounded by a thick white fluid which is used by the growing embryos. The egg (preserved material) measures 0·133 mm. across.

The newly hatched veligers measure each 0·233–0·250 mm. across the shell. The larval shell is smooth, colourless and fairly transparent. The shell-siphon is present. The animal is pale yellow. The velum is thick, bilobed and bordered with long cilia. The sub-velum is distinct with small cilia. Only the right tentacle is present and has fine hairs at the distal end. The left tentacle is not yet developed. The foot is ciliated. The two statocysts are distinctly seen. The operculum is present. The liver is brownish yellow. The alimentary tract appears bluish. The stomach is ciliated. The tissue near the anal area is brownish blue. The heart-beat is visible.

The membrane covering the exit hole gives way for the larvæ to come out. The newly hatched veligers swim about quite actively. They remained alive at the laboratory for four days but no visible changes could be noticed during the time. The well-developed velum points to the long pelagic period of larval life.

Nassidæ

The egg capsules of various species of *Nassa* are known in the literature (Lamy, 1928; Ankel, 1929; Lebour, 1931, 1937; Vestergaard, 1935; Risbec, 1935). Nothing is known of the breeding habits of *Nassa* from Indian waters.

Nassa jacksoniana (Quoy and Gaimard) (Photo 33 and Figs. 109–113).—The breeding habits of this species are described here for the first time.

This is the commonest species of *Nassa* found abundantly in certain areas in the mud flats at Kundugul Point. Large numbers of this species

were also observed at Watchman's Bay on Krusadai Island and Pamban (Satyamurti, 1952). At low tide patches of water are left behind here and there while large areas are exposed in the mud flat and on the edge of these small puddles of water the adult specimens are found in large numbers moving about actively. These forms are easily kept at the laboratory for three to four months; only it requires changing of water once in two or three days.

The snails deposited the egg capsules on the sides of the glass trough in the months of January (salinity 35·58‰, temperature 24·5° C.), February (salinity 33·12‰, temperature 26·5° C.), March (salinity 31·06‰, temperature 25·8° C.), April (salinity 32·27‰, temperature 28·2° C), May (salinity 32·36‰, temperature 28·2° C.), August (salinity 35·68‰, temperature 26·5°C.) and December (salinity 29·07‰, temperature 25·0° C.). It is very likely that the breeding in this species occurs all the year round. The egg capsules could not be collected from the field in spite of close examination of the possible substrata like the shells, seaweeds, etc., the main disadvantage being the smallness of the capsules. It is very likely that the breeding of this species in the field is identical as in the laboratory.

In this species, the egg capsules are laid singly, as in *Nassa incrassatus* (Cooke, 1895; Lebour, 1931). The capsules laid in glass trough are close to the surface of the water or below about the middle of the glass sides but not on the bottom. The animal lays conical capsules on a disc-shaped plate. This ground plate is whitish and transparent and helps in fixing the capsule to the substratum. This feature is identical with that described for *Nassa mutabilis* (Korschelt and Heider, 1936). The capsule has one side flattened, *i.e.*, the side which is laid on the ground plate. The other side has a wrinkled surface and is colourless and transparent revealing clearly the developing embryos inside. The neck of the capsule is well defined and measures 0·116–0·183 mm. in length. The aperture of the neck at the end forms the preformed exit hole for the larvæ at the time of hatching. It measures 0·300–0·367 mm. across. It is circular or elliptical in outline. The top of the neck appears to be covered by a thin membranous lid which is thrown off at the time of hatching. The capsules laid on the sides of the glass trough have the aperture facing downwards. This will, without doubt, help the larvæ to come out quite easily. The length of the capsule (excluding the basal plate), and the largest breadth of the capsule (excluding the basal plate) are 1·00–1·65 mm. and 1·35–2·00 mm. respectively. The number of eggs per capsule is 25–72. The numbers of egg capsules laid by a few females are: 27, 25, 9, 9, 13, 6, 8 and 8 respectively. The capsules are laid very quickly; the adults do not appear to take more than 2–4 minutes for a capsule.

The eggs are yellowish, spherical and each measures 0·150–0·167 mm. in diameter. They are suspended all over inside the capsule in a colourless nutritive fluid. It takes about 4 days from the time the eggs are laid to the time of hatching. The temperate species of *Nassa* seem to take a longer duration of time, *i.e.*, 3–4 weeks from spawning to hatching (Pelseneer, 1911; Lebour, 1931). All eggs develop into embryos. Pelagic development appears to be the normal feature in *Nassa* (Lebour, 1937). But Risbec (1935) observes non-pelagic development in *N. suturalis*. In the present case, however, the development is pelagic.

The embryo examined at an early stage measures 0·154 mm. across the shell. The shell is colourless, thin and transparent and is cup-formed. The velum is colourless and measures 0·175 mm. across and is bordered with few long cilia on each side, the rest of the margin showing only small cilia. The eyes are present but both tentacles have not yet developed. The foot is ciliated. The otocysts are present. No other organs are distinguishable excepting the heart-beat.

The embryo in the hatching stage measures 0·233 mm. across the shell. The animal is pale yellowish. The bilobed velum, foot, otocysts and operculum are clearly seen through the capsule. Only the right tentacle appears to be present. The embryos are moving actively inside the capsule till they leave through the aperture of the capsule.

The newly hatched veligers are very active as soon as they leave the capsule. It has a smooth transparent and unsculptured shell. The latter measures 0·233–0·250 mm. across. The outer lip of the larval shell is slightly extended into a slender process in the middle. The velum is colourless and bilobed with long cilia bordering the margin. There is a sub-velum underneath with small cilia. In between there is a groove in which the food particles are collected and brought to the mouth. The eyes are black and distinct and only the right tentacle is well developed and the left one is comparatively small. Pelseneer (1911) observes such a feature of disparity of tentacles in *Nassa reticulatus*. The otocysts, foot and operculum are quite distinct. The foot is ciliated but the cilia are small and indistinct. Also it has few long hairs at its distal end. The tentacles bear a few long hairs at the tip. The heart-beat is visible through the shell. The liver is pale yellow.

The veliger thrived at the laboratory only for 3 days. During this period no visible changes could be noticed. The well-developed velum bordered with long cilia points to a long pelagic life for the larva.

Nassa costata Adams (Photos 34 & 35 and Figs. 114–119).—The breeding habits of this species are described here for the first time.

This species is found in large numbers in the mud flats at Kundugal Point and in certain places at Vedalai. Satyamurti (1952) has also observed this species from Krusadai Island. This species like *Nassa jacksoniana* has been kept at the laboratory for several weeks without difficulty. They spawned at the laboratory in the months of January (salinity 29·61‰, temperature 24·0° C.), February (salinity 31·94‰, temperature 26·5° C.) and March (salinity 31·06‰, temperature 25·8° C.). It is very likely that the breeding in this species may occur all the year round as in the previous species. Both these species of *Nassa* are found in the same habitat.

The capsules are laid in rows. Number of such rows are laid parallel and close to each other. The adhesive bases of these capsules are confluent so that a single sheet of common base is formed. The entire structure looks like a slender ribbon with fine strands, each strand representing a row of capsules. These slender ribbons give off branches in a characteristic way on either side which may again ramify into finer branches. This pattern of arrangement of egg capsules has not been met with in any species of *Nassa* whose breeding habits are known. Unfortunately the entire structure of the egg mass could not be photographed as the ribbons are so delicate that they come off in bits while removing from the substratum.

The capsules appear to be made up of two convex faces which are fused on sides as well as on the top, leaving a broad edge on the top. On the proximal end they are joined by a basal plate which forms the base of the capsule. The capsules are more or less triangular in outline. There is no stalk. The wall of the capsule is colourless, smooth, thin and transparent revealing the egg inside. Each capsule contains only one egg unlike the previous species. There is no preformed exit hole or aperture. The shape of the capsule as well as the presence of only one egg per capsule recall Risbec's account (1935) of *Nassa suturalis*. In the latter species there is a long well-developed stalk, unlike the present one. The length, the breadth at the distal end and the breadth at the proximal end of the capsule are 0·45–0·55 mm., 0·35–0·55 mm. and 0·10–0·20 mm. respectively.

The three egg masses examined contained 671, 1,652 and 1,009 egg capsules respectively. The egg is yellowish, spherical and is placed in the centre of the capsule. It measures 0·200 mm. across. It is surrounded by a white albumen-like substance. The time of development from spawning to hatching is about 4 days.

The embryo just before hatching measures 0·283 mm. across the shell. The larval shell is colourless, sculptureless and transparent. The velum is bilobed and measures 0·250 mm. across. Eyes are black. Both the tentacles

are seen. Foot, operculum and otocysts are present. The foot is ciliated. The internal organs are not yet clearly distinguishable.

The day-old veliger measures 0·317 mm. across the shell which shows one whorl. The outer lip of the shell is extended forward in the middle. The velum measures about 0·300 mm. across. The sub-velum is present with small cilia. The tentacles are well developed and show a few long hairs at the tip. The foot is extended in the middle at the free end into a tentacular process which shows long hairs at the tip. The course of the alimentary tract is clearly seen. The anterior part of the stomach is slightly purplish. Liver is yellow. Retractor is visible.

The seven-day old veliger measures 0·400 mm. across the shell which is colourless and fairly transparent. The margin of the aperture is brownish. The outer lip of the shell is prominently extended in the middle. The shell-siphon is present. The velum is very well developed and now measures 0·551 mm. across. The length of each velar lobe is 0·334 mm. The tentacles are well developed. Eyes are black and prominent. The veliger thrived at the laboratory for 8 days.

Nassa thersites (Bruguière) (Photos 36 & 37 and Figs. 120 & 121).—The breeding habits of this species are described here for the first time.

This species is quite common in the Pamban area and numerous live specimens have been collected on the mud flats at Kundugal Point. They are also found on the sandy stretches at Watchman's Bay and Bushy Point on Krusadai Island. This species have been reared in the laboratory for months. About 20 adult specimens kept in the aquarium thrived for more than 7 months. They spawned at the laboratory in the months of July (salinity 33·46‰, temperature 27·0° C.) and August (salinity 33·86‰, temperature 27·5° C.).

In the laboratory the egg capsules were laid on the glass wall of the aquarium or upon the live or dead shells of *Cerithidea fluviatilis*. The four egg clusters examined contained 21, 33, 30 and 64 capsules respectively. The egg clusters are laid in patches consisting of a few rows of capsules. Occasionally they are also laid singly. Each capsule appears to be made up of two convex faces which are fused on the sides as well as on the top as in *Nassa costata*. The capsule is broad at the top and gradually tapers downwards. There is no exit hole or aperture in the capsule. The capsule is white, smooth and transparent and contains only one egg. Each capsule is provided with a well-developed base by which the capsule is attached to the substratum. The base is thin and transparent. There is no stalk for the capsule. The length, the breadth of the capsule at the distal end, and the breadth of the

capsule at the proximal end are 0·46–0·52 mm., 0·43–0·60 mm. and 0·23–0·32 mm. respectively.

The egg is yellowish and placed in the centre of the capsule. It measures 0·250 mm. across. It is surrounded by a white transparent nutritive fluid. The time of development from spawning to hatching is about 4 days in the laboratory. The embryo in the hatching stage shows smooth, colourless and sculptureless shell of a diameter of 0·317 mm. The velum, eyes, tentacles, otocysts, foot and operculum are clearly seen. The other details are not clear. The newly hatched veliger measures about 0·350 mm. across the shell. The shell is still sculptureless. The animal is pale yellowish. The velum is colourless and well developed and has two equal lobes bordered with long cilia on the margin. Underneath the velum and close to its margin is present the sub-velum with short cilia. The eyes are black. The tentacles are long and very prominent with the tip bearing long hairs. They are of equal size unlike in *N. jacksoniana*. The foot is ciliated but the cilia are vague. It is extended distally into a slender process which bears at its tip long hairs. The operculum is colourless. The otocysts are clearly seen. The alimentary tract is pale purplish and hence could be clearly seen through the shell. The liver is yellowish. The heart-beat is visible. The veliger thrived at the laboratory for two days. The larva did not show much change from the newly hatched veliger excepting a slight growth in the larval shell, which measures 0·434 mm. across. The outer lip of the shell is slightly extended in the middle and tends to curve inwards. It shows a brownish or pinkish brown tint and fine dots, the latter are visible only under high power. The velum measures 0·418–0·467 mm. across. The well-developed velum denotes a long pelagic life for the larva.

Olividæ

No details regarding the breeding habits of the members of this family seem to be available in the literature.

Ancilla sp. (Photo 38 and Figs. 122–125).—The present egg masses which appear to belong to the genus *Ancilla* have been collected from Kundugal Point in August and September. The capsules are laid on the surface of polychæte tubes. The entire egg mass is below the level of the soil. The capsule has a flat bottom with a distinct margin. It projects upwards in the form of a cone. The cone-like capsule appears to be made up of two distinct halves which are fused in the middle. The line of fusion which is very distinct breaks apart at the time when the young ones are ready to come out. The capsule is parchment-like and quite smooth with the largest diameter of 4 mm., smallest diameter of 3·5 mm. and height 2·5 mm. It holds only

one embryo surrounded by a thick white substance which is probably utilised by the developing embryo. Here the entire development including the larval stages takes place within the capsule and the young one comes out in the crawling stage. Thus the development is non-pelagic.

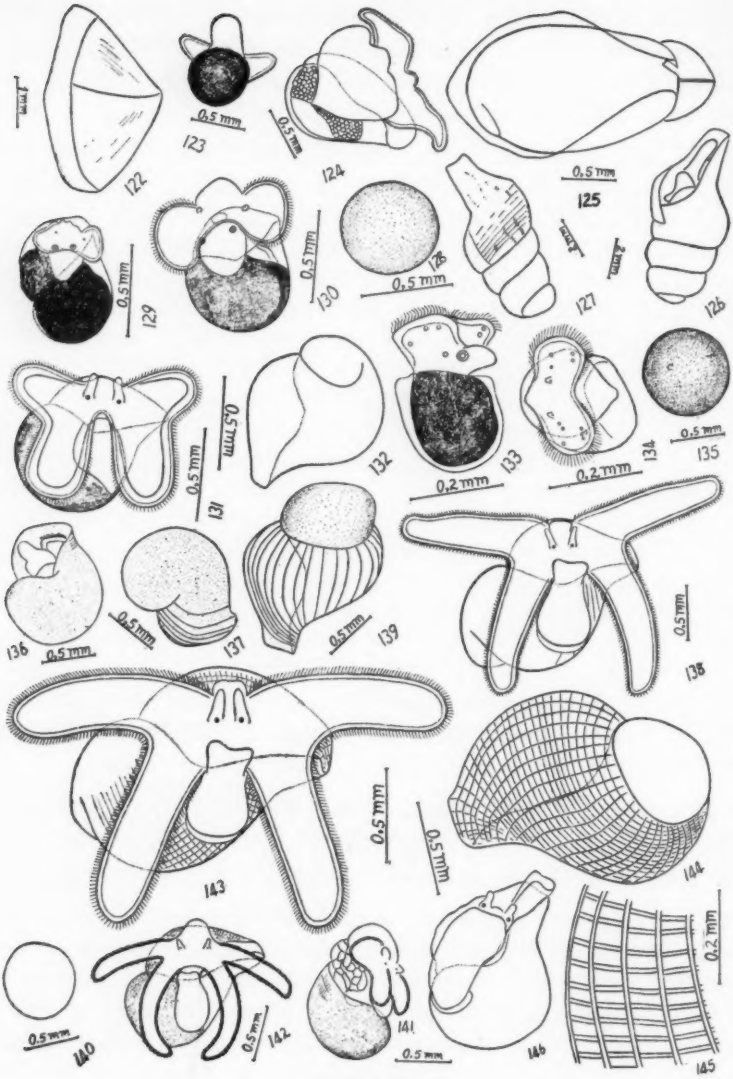
In the early stage the embryo measures 0·65 mm. The shell is cup-formed. The velum is represented by two appendages with the margin showing small cilia. Foot is present. The embryo itself is little differentiated and apparently only contained a yolky mass. At a later stage the embryo shows the characteristic shell which is elongate with a long narrow aperture. It does not show any sculpture and is whitish and smooth. It measures 1·169 mm. The foot is thick and very well developed and measures 0·70 mm. The velum is more or less a narrow colourless band bordered with small cilia. It measures 1·33 mm. In the next stage the embryo loses the velum and comes out as the young gastropod. The young ones show a well-developed foot with more or less semi-lunar propodium with a longitudinal groove in the middle. The shell shows further growth and measures 1·90 mm. The siphon is present. The animal crawls about slowly. It could not be kept at the laboratory for more than 24 hours. The time of development from the egg to the hatching could not be given but the egg mass that was collected on 8th August 1952 liberated the young ones on 20th August 1952.

Vasidæ (*Turbinellidæ*)

Information on the spawn masses of the members of this family is available in the literature (Lamy, 1928; Risbec, 1931; Hornell, 1921; Chidambaram and Unny, 1947).

Xancus rapa (Lamarck) (Photo 39 and Figs. 126 & 127).—Egg cases belonging to this species have been collected from Vedalai and Mandapam areas in the month of January, February, April, July and August.

The egg case is an elongated, loosely spirally twisted structure and is aptly compared to a miniature ram's horn by Hornell (1921). It has a base by which it is attached to the sandy substratum. Each egg case is really a compound capsule as it is made up of distinct chambers or capsules. The chamber has a dome-shaped upper part and a thin-walled floor which is surrounded by a broad edge. Each chamber is a separate unit placed one above the other. They are attached to a membrane on one side and free on the opposite side. The floor of one capsule fits in compactly on the dome of the lower capsule. In the floor of each chamber is present a crescentic or nearly straight slit. This slit is probably the preformed exit passage. It varies in length and



FIGS. 122–146

measures 5–15 mm. It is covered by a thin transparent membrane. The chambers are not of uniform size. The chambers at the proximal end are small and increase gradually in size upwards till about $\frac{3}{4}$ of the egg case and afterwards there is a slight decrease (not very appreciable) in size up to the

end. Usually 7–8 chambers from below and 2–4 from above do not hold any embryos. The breadth of the chamber varies between 1·3 and 2·8 cm. The length measurements of three egg cases are 22 cm., 28·2 cm., and 29·5 cm. respectively. The total number of chambers for three egg cases are 28, 33 and 36.

Egg cases at early stages of development could not be collected. On an average, each egg case produces 122 surviving young ones. The young ones at the time of examination showed about 4 whorls. The first 3½ whorls form the larval shell and the rest beginning to show the adult features. The first 3½ whorls do not show any sculpture. They are smooth, plain and colourless. The last whorl begins to show few pinkish brown spots. It also shows very feeble spiral striations. The shell-siphon is distinct. The aperture is elongate. In the specimens studied the young ones were in the withdrawn condition with the foot slightly pushed out. The operculum is brownish in colour. The foot also shows some pinkish spots. The shell measures about 9 mm. in length and 3 mm. in breadth. The young ones thrived at the laboratory for a day.

Conidæ

The egg masses of several members of this family have been described from other countries (Lamy, 1928; Petit and Risbec, 1929; Risbec, 1931, 1932; Thorson, 1940; Lebour, 1945; Knudsen, 1950; Ostergaard, 1950). Not much is known of the breeding of *Conus* from Indian waters excepting brief accounts by Hornell (1922) and Gravely (1942).

Conus araneosus Hwass. (Photos 40 & 41 and Figs. 128–32).—The breeding habits of this species are described here for the first time.

This species appears to be quite common at Ceylon from where numerous shells are obtained to be sold as ornamental paper weights. It has been recorded at Pamban (Satyamurti, 1952). Numerous live specimens of this species have been collected from the Gulf of Mannar at Vedalai, Seeniyappa Dharuga and Mandapam. The adult specimens laid the egg masses at the aquarium on 25th January 1955. They were found attached to the glass of the aquarium close to the bottom, the temperature and salinity being 24·8° C. and 29·22‰ respectively. The animal of a length of 4·7 cm. and breadth of 2·7 cm. took about 2 days to lay an egg mass of 45 capsules.

Gravely (1942) figures a mixed egg cluster from Madras probably laid by two closely allied species. He observes that they may belong to the genus *Conus* or to one of the other families of Toxoglossa. The figure as well as the few details given by him agree broadly with the present kind of egg mass and hence may be referred without doubt to a species of *Conus*.

The breeding of the present species has been observed in the months of October, November, December, January, February and March.

The egg clusters collected from the field (Vedalai) were not attached to any substratum at the time of collection. Each capsule is a compressed pouch. It is white or pale-yellow. It is slightly curved, the bend being more pronounced generally towards the lower part of the capsule. The convex face of the capsule appears rugged made up of a number of elevations and depressions. There are also two or three fairly pronounced ridges on the same face running from the top towards the base. On the concave side there are two somewhat inwardly curved ridges that extend downwards from the upper angles. These ridges, however, are vague. Excepting these ridges this face is plain and smooth. The developing embryos are seen clearly through the exit passage which is located on the top of the capsule in the form of a slit and is covered by a thin, transparent, hyaline membrane. It almost occupies the entire top of the capsule and measures 4–6 mm. This type of exit passage very nearly corresponds to that of *Conus mercator* (Knudsen, 1950). The hyaline membrane dissolves at the time of hatching of the larvæ. The wall of the capsule is semi-transparent and gives a blurred view of eggs or embryos inside. It has a base which is very thin and papery and measures 8–11 mm. across. The length as well as the largest breadth of the capsule are 9–17 mm. and 5–11 mm. respectively. The number of embryos per capsule is 63–214. The average length, breadth and number of eggs for a capsule are 14·5 mm., 9 mm. and 164 respectively. The egg mass appears outwardly to consist of a bunch of compact capsules. On a closer scrutiny these capsules appear to conform to some pattern of arrangement. Thus there appears to be a bottom layer of capsules. These capsules bear on their convex surfaces a second layer of capsules, which in turn bear the third layer and so on.

The unsegmented egg is spherical and yellowish measuring 0·467–0·517 mm. across. The eggs are scattered all over inside the capsule, being suspended in a white slimy nutritive fluid. The growing embryos not only make use of the nourishment contained in the eggs but also seem to devour the nutritive fluid surrounding them and grow to a remarkable size.

Among the species of *Conus* the development may be pelagic or non-pelagic, thus pelagic in *C. hebræus*, *C. capitaneus* and many others (*cf.* Risbec, 1931; Thorson, 1940; Lebour, 1945) and non-pelagic in *C. mercator* (Knudsen, 1950). In the present species, however, it appears to be pelagic. The embryo examined at the early stage measures 0·651 mm. across the shell. The velum is very small and bilobed and measures 0·334 mm. across. It is bordered with small cilia at the margin. It is pushed towards one side. The

eyes and otocysts are present. The tentacles are not yet developed. The foot is small. The larva is little differentiated. The embryo examined a few days later showed the well-developed velum. The shell measures about 0·684 mm. across. It is colourless and sculptureless with the shell-siphon just appearing. The velum is bilobed, the lobes being almost equal. It now measures 0·617 mm. across. The sub-velum is not yet distinct. The eyes and tentacles are present though not well developed. The foot and operculum are present. The otocysts are quite small but fairly clearly seen. The embryo itself is still little differentiated. The anterior part of the alimentary tract is vaguely seen. Heart-beat is visible.

The larva at the next stage is further developed in many respects. The larval shell shows further increase in size measuring 0·800–0·850 mm. across. The velum has grown still bigger and measures 0·935 mm. across. It has a tendency to form four lobes. The sub-velum is now clearly seen. The eyes and tentacles are more prominent. The foot and operculum also show increase in size correspondingly. The otocysts are not clearly seen as in the younger stage. The internal organisation of the larvæ is not yet differentiated.

The hyaline membrane covering the exit passage gives way at the time of hatching. The embryos did not hatch out at the laboratory condition. The larvæ about to hatch out were dissected out from the capsule and kept in the finger bowl. They thrived for 36 hours. The larval shell now measures 1·10 mm. and the velum 1·75 mm. The velum is now distinctly four-lobed. The foot also shows increase in size comparatively. The larvæ swim as well as crawl, the latter feature probably denotes a short pelagic period.

Conus sp. (Photos 42 & 43 and Figs. 133 & 134).–The present egg clusters have been referred to the genus *Conus* on the basis of the identification of similar capsules by Gravely (1942) from Madras. They could not be referred to the species with certainty. They have been collected from the mud flats at Kundugal Point in the month of August.

The capsules are strung together into a bunch but conform to a particular pattern of arrangement. The arrangement in one of the egg clusters is as follows: One of the capsules forms more or less the substratum for the entire bunch of capsules. This bears four capsules on one of its faces. Only two capsules of the four bear a batch of capsules on their faces, one of them bearing a row of four and the other seven. Again only one of the seven capsules bears a row of five capsules. Thus altogether there are 21 capsules. Similar arrangement with slight modifications has been found in the other egg cluster consisting of 13 capsules. The egg clusters of this species thus differ very much in the arrangement of capsules from those of *C. araneosus* whose pattern

of arrangement is altogether different. The arrangement of capsules in the latter makes the egg cluster quite compact unlike the present one where it is loose.

The capsules which are compressed and pouch-like are strung into a bunch. They are pale yellowish in colour. Each capsule has a well-defined base. The base is thin and papery. The length of the capsule is 3–3·2 cm. and largest breadth 1·8–2·1 cm. The capsule is covered by a network of ridges which are found on both faces. A pair of ridges on one face of the capsule but not on the other and quite distinct from others extend obliquely downwards from the upper angles. The exit passage is not preformed but the top of the capsule slits open at the time of the emergence of the larvæ. This slit or the exit passage measures 1·4–1·8 cm. The number of eggs per capsule seems to be in hundreds (the exact number could not be given) and they are suspended all over inside the capsule in the colourless slimy fluid. All the eggs develop into embryos.

The egg clusters which were collected on 8th August 1952 liberated the veligers on 15th August. The embryo in the hatching stage measures 0·200–0·233 mm. across the shell which is cup-formed. The velum is bilobed, bordered with small cilia. It shows a few greenish pigment spots in each lobe. Foot and otocysts are clearly visible but eyes and tentacles are not seen. The embryo is little differentiated and apparently only contained a yolky mass. The veliger of two days old shows a well-developed bilobed velum, the lobes being almost equal in size and shape. The velum measures 0·250 mm. across. The greenish pigment dots in each lobe are more prominent. Eyes and tentacles are now seen. The foot and operculum are present. The otocysts are indistinct unlike in the previous stage. The shell is thin and fragile and measures 0·250 mm. across. The internal organs of the larvæ are not yet distinguishable excepting for the heart-beat. The veliger could not be kept at the laboratory for more than 4 days and during this period no further changes in them could be noticed. Larvæ may have a long pelagic life as is seen from the size of the velum.

Egg capsule A. (Photos 44 & 45 and Figs. 135–139).—The egg capsules of the present type have been referred to the genus *Conus* by Gravely (1942) and *Hemifusus* by Habe (1944). Further investigation is required to refer the egg mass with any certainty to the genus and species.

The egg clusters have been collected from Gulf of Mannar around Mandapam, Seeniyappa Dharuga and Vedalai in the months of October (temperature 28° C., salinity 36·76‰), November (temperature 27° C., salinity 32·54‰), December (temperature 26·1° C., salinity 29·69‰), January

(temperature 24° C., salinity 30·75‰), February (temperature 25·3° C., salinity 29·33‰), and March (temperature 27·3° C., salinity 31·42‰). They were usually found attached to the shells of the live specimens of *Xancus rapa*. The six egg clusters examined showed 41, 45, 29, 25, 34 and 37 capsules respectively.

The capsule is compressed and pouch-like. It has fairly well pronounced depression on one side in the middle towards the upper end bounded by two well-pronounced inwardly curved ridges which extend downwards from upper angles. The other face is smooth without ridges. The top is slightly bowed seen from above. The wall of the capsule is tough, pale yellowish and semi-transparent. The compressed capsules are placed side by side with the broad sides opposite and parallel to each other. The measurements of the capsule are the following: total height (from the substratum to the extremest part of the top border) 19–30 mm.; height of the peduncle 1·75–4·25 mm.; largest breadth of the capsule 14–20 mm.; smallest breadth of the capsule 5·9 mm. Each capsule has a distinct base by which they are glued to the substratum. The bases of the capsules are usually laid so close to each other that they form a long narrow sheet from which the capsules seem to arise. The top of the capsule has a well-defined preformed exit hole which is covered by a thin membrane. The exit hole is oblong or elliptical and measures 2–2·5 mm. across. The number of embryos per capsule is 54–119.

The unsegmented eggs are quite large, spherical and yellowish, each measuring 0·700–0·750 mm. across. Almost in each capsule one to few eggs remain undeveloped. The eggs are scattered all over inside the capsule, being suspended in a colourless slimy fluid—probably consisting of albumen. The embryo, without doubt, devours the fluid and grows inside the capsule to a size much larger than that for which the nourishment contained in the egg itself could suffice.

The embryos did not hatch out in the laboratory condition. However the larvæ in the hatching condition were dissected out from the capsules and kept in finger bowl for examination. They measure 0·95–1·45 mm. Of this range, the larvæ measuring 1·25–1·45 mm. form 85% and appear to be normal development. Comparatively judging the larvæ measuring 0·95 mm. appear to be sub-normal and form about 1% of total larvæ on an average. It is not possible at the moment to attribute any reasons for the apparent disparity in growth among the larvæ.

The larva which measures 0·95 mm. has just more than one whorl. The velum appears to be bilobed with a slight indentation in the middle. The

eyes and tentacles are not clear. The foot and operculum are seen. The embryo is little differentiated.

The larvæ measuring 1·25–1·45 mm. across the shell have each about 2 whorls, the second whorl showing characteristic sculpture consisting of longitudinal lines. The first whorl is sculptureless. The shell also shows a well-developed shell-siphon. The velum is distinctly four-lobed and colourless, each lobe being long and narrow. The cilia bordering the margin of the velar lobes are quite short. The eyes and tentacles are present but appear quite small. The otocysts are not distinct. The foot and operculum are present, the former being slightly darkish. The internal organs of the larvæ are not yet clear. Heart-beat is visible. The larvæ swim as well as crawl, the latter feature denoting their short pelagic life. The larvæ thrived at the laboratory for about 12 hours.

Egg capsule B. (Photos 46 & 47 and Figs. 140–146).—The egg masses of this species have been observed at Vedalai, Seeniyappa Dharuga and Mandapam in the months of December, January, February and March. It appears that the breeding in this species may begin from October as in the previous species and range for six months.

The egg capsules were generally found attached to the shells of the live specimens of *Xancus rapa*. Occasionally wooden planks or twigs blown into the sea from the land also form the substrata. The nine egg clusters examined show 27, 33, 54, 31, 27, 25, 24 and 28 capsules respectively. The capsule is compressed and pouch-like and is uniformly flat except for a depression in the middle on one face towards the top end bounded by two distinct inwardly curved ridges which extend downwards from upper angles. One of the incurving ridges is slightly longer than the other. There are no ridges on the other face of the capsule. The top of the capsule is not straight but slanting as seen from above. The capsules are pale yellowish and semi-transparent. The measures of the capsule are the following: Total height (from the substratum to the extremest part of the top border) 16–20 mm.; height of the peduncle 2·25–3·25 mm.; largest breadth of the capsule 7–9 mm.; smallest breadth of the capsule 2·5–4 mm. Each capsule has a well-developed base. A well-defined preformed exit hole is present on the top border covered by a thin membrane. It may be close to the centre or towards one side (inside the ridge). The exit hole measures 1·5–1·9 mm. The number of embryos per capsule is 39–84.

The eggs (undeveloped ones) are spherical, yellowish and quite big each measuring 0·601 mm. They are scattered all over inside the capsules in a

white slimy fluid which is probably of nutritive nature. In each capsule one or two eggs remain undeveloped. The embryos reach a remarkable size at the time of hatching.

The embryo examined on the 11th day after oviposition has shell which is about a whorl and measures 0·801 mm. across. The larval shell begins to show some spiral as well as transpiral striations. The velum is colourless and bilobed but shows a tendency towards four lobes. The cilia bordering the velar lobes are very small. Eyes are small. Tentacles are seen. Foot and otocysts are present. The larva is little differentiated. The heart-beat is visible. The animal is yellow.

The embryo examined on the 18th day after oviposition has a velum which is four-lobed each lobe being long and narrow. Eyes and tentacle are seen. Foot is little blackish. Otocysts are present. The shell-siphon is present. The heart-beat is visible. The internal organs are not clear. The embryo is yellowish.

The larvæ about to hatch out were dissected out from the capsule and kept in the finger bowl for examination. They measure 1·10–1·43 mm. across the shell. Of this about 90% of the larvæ measure 1·32–1·43 mm. The smallest observed was 0·85 mm. This forms a very small and negligible percentage. The larvæ measuring 1·32–1·43 mm. have each two whorls. The second whorl has a characteristic sculpture consisting of longitudinal as well as spiral striations. The first whorl is almost sculptureless. The velum is well developed and colourless. It consists of four distinct velar lobes, the lobes being long and narrow. The cilia bordering the margin of the velar lobes are quite short. The eyes and tentacles are present. The foot and operculum are distinct. The otocysts are not clear. The larva is little differentiated yet and has a tendency to swim as well as crawl. The latter feature indicates the short pelagic life of the larva.

These larvæ lose their velar lobes and settle down in 2–3 days in the laboratory. The 8-day-old young one measures 1·45–1·50 mm. across the shell. The shell is slightly brownish and tentacles are prominent. Foot is well developed. The young ones crawl about the finger bowl. They thrived at the laboratory for 25 days. But no noticeable growth could be observed during the time.

4. Discussion

Oviposition.—The egg masses of marine prosobranchs are very characteristic in their shape and appearance. The following types have been met with in the present investigation: gelatinous ribbons (*Cerithidea fluviatilis*, *Ceri-*

thium morus), peculiar sand encrusted collar-like structures (*Natica marochiensis*), large slimy wavy bands (*Tonna dolium*) and capsules of various shapes and sizes (*Murex virgineus* var. *ponderosa*, *Thais bufo*, *Nassa jacksoniana*, *Ancilla* sp., *Conus araneosus*, etc.). In the holo-pelagic *Ianthina prolongata*, the egg capsules are suspended underneath the float. The capsules are usually parchment-like but in the genus *Nerita* (*N. albicilla* in the present study), they are calcareous (excepting the base which is membranous). In primitive prosobranchs like *Cellana* sp. (not included in the present paper) the eggs are extruded singly in hundreds and fertilisation is external. There are also known in the literature cases of viviparity. Brood protection has been noted in the cowry *Erronea errones*. This appears to be the case in all the true cowries. In the nun cowry, *Trivia monacha*, the animal bites holes in the compound ascidians into which the vase-shaped egg capsules are embedded (Lebour, 1937).

Larval feeding and development.—Embryos with non-pelagic development may feed in different ways. Thus the large yolky eggs sufficiently rich in food may support the whole development up to the bottom stage, *e.g.*, *Lacuna pallidula* (Pelseneer, 1911). In others the yolky eggs are further surrounded by a thick sheet of nutritive substances as in *Ancilla* sp. of the present study. In all species with these types of feeding the embryonic development seems to be uniform in that the embryos leave the egg capsules after reaching a certain size and organisation. Thus all the hatching young of the same species will be at the same stage of development. This, however, is not the case when the embryos resort to nurse egg feeding. Eggs of more or less uniform size are laid together within a common egg space or capsule. Only some of the eggs develop into embryos while the rest subserve as nurse eggs for the developing ones. The latter will not hatch out until they have exhausted all the nurse eggs. Nurse eggs feeding need not necessarily lead to a non-pelagic development. This is exemplified by the present example of *Murex virgineus* var. *ponderosa*. It is now well known that the pelagic stage is the most sensitive period in the life-cycle of marine invertebrates and consequently large waste of larvæ occurs during this period (Thorson, 1946). This shows clearly the evolutionary significance of nurse egg feeding (Thorson, *l.c.*). The eggs which could develop into pelagic larvæ in primitive times under favourable conditions may under adverse conditions have differentiated into nurse eggs (probably unfertilised ones) and normal eggs, the former subserving as food to the latter, so that the embryos can spend the entire veliger stage (pelagic) inside the capsule and come out as young ones or they emerge out as advanced veligers for a short pelagic life.

It has been found in the Arctic marine prosobranch *Sipho islandicus* that the capsules with nearly the same quantity of nurse eggs may contain from 1–16 embryos. Naturally in the capsule holding 16 embryos, the embryos hatch out at a much earlier stage (though non-pelagic) than a single embryo contained in a capsule with plenty of food at its disposal (Thorson, 1935). *Natica catena* provides a fine example in which the development is pelagic at one place, *i.e.*, Southern North Sea (Lebour, 1936) and non-pelagic at another place, *i.e.*, Denmark (Thorson, 1946), in each case the development being determined by the amount of nurse eggs. *Planaxis sulcatus* reproduces without nurse eggs and with pelagic larvæ at New Caledonia (Risbec, 1935) and with nurse eggs and without pelagic development in the Iranian Gulf (Thorson, 1940). Similar is the case in *Thais hæmastoma* (*T. floridana*) which reproduces without nurse eggs and with pelagic larvæ in Louisiana (Burkenroad, 1931) and with nurse eggs with non-pelagic development in West Indies (Lamy, 1928; Korschelt and Heider, 1936). A phenomenon of more or less similar type seems to exist within the genus *Dolium*. In *Tonna dolium* (*Dolium maculatum*) observed here, there seems to be no nurse egg feeding, as all the eggs develop into pelagic larvæ. Though similar mode of reproduction appears to be the case in this species from Iran, Thorson (1940) finds in a few egg spaces considerably large embryos which he suspects as may be due to nurse egg feeding or cannibalism.

The availability of nurse egg per embryo varies from a few to thousands, thus a few in *Natica catena*, *i.e.*, 50 nurse eggs for 3 or 4 embryos (Ankel, 1930), 10–15 nurse eggs per embryo in *Nucella lapillus* (Lebour, 1937), 72–251 per embryo in *Murex quadrifrons* (Knudsen, 1950), about 2,500 in *Neptunea antiqua* (Thorson, 1946), about 7,000 per embryo in *Sipho islandicus* (Thorson, 1935) and 50,000–100,000 per embryo in *Volutopsis norvegica* (Thorson, 1940). In *Murex virgineus* var. *ponderosa* and *M. trapa* described here it is about 24 and 10 respectively. The method of devouring the nurse eggs is interesting in different species. Thus in *Nucella lapillus* (Thorson, 1946), each capsule contains some hundred eggs of which only a few develop, the rest forming nurse eggs. The nurse eggs fall to pieces and form a column of yolk in the middle of the capsule on which the embryos feed by sucking. In *Buccinum undatum* (Portman, 1925), as in the previous species, only a few develop out of some hundreds of eggs, the rest forming nurse eggs. The nurse eggs will not fall to pieces as in *Nucella lapillus*, but are quite intact when devoured. Knudsen (1951) finds identical phenomenon in *Murex quadrifrons* from West Africa. He observed that the embryo takes half an hour to swallow a nurse egg. In *Murex virgineus* var. *ponderosa*, a few egg capsules were cut open and the nurse eggs and larvæ were transferred to watch-glass

for observation. It was found that the eggs are manipulated by the larvæ to the mouth by velar lobes and foot and pressed. The collapsed eggs are then sucked up in 2–3 minutes. This type of nurse egg feeding seems to be different from *Nucella* and *Buccinum* types.

Influence of temperature on pelagic development.—Thorson, from his investigations at East Greenland (1935), East Iceland, South Iceland, the Feroes (1941), Danish waters (1946) and Iran (1940) as well as from other places, has been able to arrive at the conclusion that the percentage of pelagic development increases from Arctic towards Equator. Thus the pelagic development in East Greenland is 0% (Thorson, 1935); East Iceland 10%, South Iceland 30%, Faroes 40% (Thorson, 1941); British Isles 64% (Lebour, 1937); Danish waters 65%, Iranian Gulf 75% (Thorson, 1946, 1940); New Caledonia 57% (Risbec, 1935). The investigations carried out since then also seem to support this view. Thus Lebour finds pelagic development in 90% of the prosobranchs of Bermuda (Lebour, 1945). The present investigations in India show about 91% of pelagic development, *i.e.*, out of 33 species of prosobranchs [which includes *Cymbium melo* in which Hornell (1921) gives a brief account on development] only 3 species, namely *Ancilla* sp., *Xancus rapa* and *Cymbium melo*, have non-pelagic development. The prosobranchs of New Caledonia appear to show rather low percentage of pelagic development and those at Bermuda a little too high. In the case of New Caledonia, the material examined comprised mostly tidal zone forms which, generally, show a high percentage of non-pelagic development. In Bermuda the material examined is quite small and hence appears to show exaggerated percentage of pelagic development. Thorson (1940) is of the opinion that in the reproduction of prosobranchs of all oceans there is a tendency for the pelagic development to be totally suspended in the deep sea and restricted to shelf faunas, and that starting from the Arctic and Antarctic where the pelagic development is suppressed there is a gradual increase of the same towards the tropic shelves where it is the highest. The results obtained from tropical South-West Africa by Knudsen (1950) appear to present a different picture. The percentage of species having non-pelagic development in the region is about the same as in the waters of South and West Iceland. The investigations carried out along the Coast from off Sierre Leone to the Niger Delta show that this area is influenced by the Guinea current which is poor in the nutrient salts as well as plankton and brings about a strong exchange of water between the coastal and oceanic waters. It is therefore possible as suggested by Knudsen that the results obtained at South-West Africa are due to the special ecological peculiarities of the area examined.

Breeding.—Anne Stephenson (1934), Panikkar and Aiyar (1939) and Paul (1942) have shown that there are different types of both continuous and discontinuous breeding in marine and brackish-water animals in tropical waters. Even though it was not possible to maintain the breeding record of the different species of prosobranchs under investigation all the year round, it could be said from the present observations that there exist both types of continuous and restricted breeding in the forms dealt with from the Gulf of Mannar and Palk Strait. No inference could be drawn regarding the effect of temperature and salinity on the breeding. These factors appear to influence the intensity of breeding in *Ostrea madrasensis* (Rao, 1951).

5. Summary

Egg masses and larval development are described for 32 species of prosobranchs from the Palk Bay and the Gulf of Mannar. The present observations throw light on the breeding biology of *Cerithium morus*, *Cerithium* sp., *Natica tigrina*, *Natica* spp., *Tonna* sp., *Murex virgineus* var. *ponderosa*, *Murex trapa*, *Thais tissoti*, *Thais* spp., *Pyrene zebra*, *P. versicolor*, *Pyrene* sp., *Nassa jacksoniana*, *N. costata*, *N. thersites*, *Ancilla* sp. and *Conus araneosus*, which are reported here for the first time. The existing knowledge of breeding in *Nerita albicilla*, *Erronea errones* and *Pyrene flavida* has been supplemented by observations from Indian waters. In the remaining species (*Cerithidea fluviatilis*, *Ianthina prolongata*, *Natica marochiensis*, *Tonna dolium*, *Thais bufo* and *Xancus rapa*) more details on the egg structure and development are given than are available in the literature.

Breeding range of the species under investigation was determined from the observations made on the field as well as in the laboratory. Continuous as well as seasonal breeding have been noted among the species studied. Salinity and temperature were noted wherever possible but their effect on breeding requires further study.

While most of the gastropodous egg masses are left uncared for by the parents which deposit them, in the Cowry *Erronea errones* there is protection of brood until the time of hatching of the larvæ. In *Murex virgineus* var. *ponderosa* and *Murex trapa* only a few eggs develop into embryos, the rest subserving as nurse eggs. Nurse egg feeding is followed in these two species by a short pelagic life. Pelagic development is preponderant and only in 2 of the 32 species studied is the pelagic stage completely suppressed.

References*

1. Amio, M. .. *J. Shimonoseki Coll. Fish.*, 1955, **4** (2), 231–38.

2. Annandale, N. and Kemp, S. *Mem. Ind. Mus.*, Calcutta, 1916, **5**, 328–66.

3. Chari, V. K. .. *J. Bombay Nat. Hist. Soc.*, 1950, **49**, 317.
4. Chidambaram, K. and Unny, M. *Proc. Zool. Soc. London*, 1947, **117** (2/3), 528–32.
5. Cooke, A. H. .. *Camb. Nat. Hist. Moll.*, 1895.
6. Crichton, M. D. .. *J. Bombay Nat. Hist. Soc.*, 1941, **42**, 323–41.
7. Franc, A. .. *J. Conchyliol*, 1948, **88** (1), 12–35.
8. Giglioli, M. E. C. .. *J. Fish. Res. Bd. Canada*, 1955, **12** (2), 287–327.
9. Gravely, F. H. .. *Bull. Mad. Govt. Mus.* (*Nat. Hist.*), 1942, **5** (2).
10. Habe, T. .. *J. Conchology*, 1944, **13** (5–8), 171–206.
11. ———— .. *Publ. Seto Mar. Biol. Lab.*, 1953, **3** (2), 161–67.
12. ———— .. *The Venus; Jap. Jour. Mal.*, 1955, **18** (3), 204–07.
13. Harvey, H. W. .. *Recent Advances in the Chemistry and Biology of Sea-water*, 1945.
14. Hornell, J. .. *Mad. Fish. Bull.*, 1921, **14**, 97–210.
15. Kundsen, J. .. *Sci. Res. Dan. Exp. Coast of Trop. West Africa. Atlantide Rep.*, 1950, 85–128.
16. Knudsen, M. .. *Hydrographical Tables*, 1901.
17. Korschelt, E. und Heider, K. *Vergleichende Entwicklungsgeschichte der Tiere*, 2 *Band.* (New bearbeitet von E. Korschelt), 1936.
18. Lamy, E. .. *J. de Conchyliologie*, 1928, Ser. 4, **72**, 25–52, 80–126.
19. Laursen, D. .. *Dana-Report*, 1953 (38).
20. Lebour, Marie, V. .. *J. Mar. Biol. Assoc.*, 1936, **20** (3), 547–66.
21. ———— .. *Ibid.*, 1937, **22**, 105–66.
22. ———— .. *Proc. Zool. Soc.*, 1945, Pt. IV, **114**, 462–89.
23. Natarajan, A. V. .. *Curr. Sci.*, 1954, **23** (7), 225–26.
24. Ostergaard, J. M. .. *Pacific Sci.*, 1950, **4** (2), 75–115.
25. Panikkar, N. K. and Aiyar, R. G. *Proc. Ind. Acad. Sci.*, 1939, **9 B**, 343–64.
26. ——— and Tampi, P. R. S. *J. Bombay Nat. Hist. Soc.*, 1949, **48** (3), 598–99.
27. Paul, M. D. .. *Proc. Ind. Acad. Sci.*, 1942, **15**, 1–42.
28. Petit, G. *et* Risbec, J. .. *Bull. Soc. Zool. Franc.*, 1929, **54**, 564–70.
29. Rao, K. V. .. *Proc. Ind. Acad. Sci.*, 1951, **33**, 231–56.
30. Rasmussen, Erik. .. *Vid. Medd. Dansk. Naturh. Foren*, 1944, **107**, 207–33.
31. ———— .. *Ibid.*, 1951, **113**, 201–49.
32. Ray, H. C. .. *J. Bombay Nat. Hist. Soc.*, 1951, **49** (4), 663–69.
33. Risbec, J. .. *Bull. Mus. Hist. Nat., Paris*, 1928.
34. ———— .. *Ann. Inst. Ocean.*, 1931, **10** (2), 23–33.
35. ———— .. *Bull. Soc. Zool. Franc.*, 1932, **57** (4), 358–75.
36. ———— .. *Ibid.*, **60** (5), 387–417.
37. Sadasivan, V. .. *Proc. 35th Ind. Sci. Cong. Abstracts*, 1948, 198.

38. Satyamurti, T. .. *Bull. Mad. Govt. Mus.*, 1952, **1** (2), pt. 6.

39. Stephenson, A. .. *Great. Barr. Reef. Exped. Sci.*, 1934., Rep. 3, 247–72.

40. Thiele, J. .. *Handbuch der systamatischen Weichtierkunde*, 1931.

41. Thorson, G. .. *Medd. Om. Gronland*, 1935, **100** (5), 1–71.

42. ———— .. *Danish Scientific Investigations in Iran*, 1940, pt. II, 159–238.

43. ———— .. *Marine Gastropoda Prosobranchiata. Zoology of Iceland*, 1941, **4**, pt. 60, 159.

44. ———— .. *Medd. Komm. f. Danmarks Fiskeri og. Havunders*, 1946, Serie: Plankton, Bind 4, Nr. 1.

45. Vayssière, A. .. *Ann. Mus. Hist. Nat. Marseille, Zool.*, 1923, **18**, 1–120.

46. ———— .. *Ann. Mus. Hist. Nat. Marseille*, 1927, **21**, 133–84.

* For other references, not included here but cited in the text, the reader is requested to consult the papers of Lamy (1928), Lebour (1937), Thorson (1940, 1946) and Knudsen (1950).

Explanation of Text-Figures

Text-Figs. 1–18. Figs. 1–6. *Nerita albicilla*. Fig. 1. Egg capsule. Fig. 2. Sculpture of the egg capsule enlarged. Fig. 3. Egg. Fig. 4. Newly hatched veliger, retracted into the shell. Fig. 5. Newly hatched veliger, swimming posture. Fig. 6. Day-old veliger, retracted. Figs. 7–9. *Cerithidea fluviatilis*. Fig. 7. Egg capsule showing the egg inside. Fig. 8. Embryo in the hatching stage with the velum and foot partly retracted into the shell. Fig. 9. Larval shell of the newly hatched veliger. Figs. 10–18. *Cerithium morus*. Fig. 10. Egg capsule showing the egg inside. Fig. 11. Embryo in the hatching stage. Fig. 12. Larval shell (left side) of the same. Fig. 13. Veliger larva, 1 day-old. Fig. 14. The same with the animal completely retracted into the shell. Fig. 15. Larval shell of the same (right side). Fig. 16. Veliger larva 4-day old. Fig. 17. Larval shell (right side) of the same. Fig. 18. Larval shell (left side) of the same.

Text-Figs. 19–40. Figs. 19–22. *Cerithium* sp. Fig. 19. Egg capsule showing the egg inside. Fig. 20. Newly hatched veliger with the velum and foot partly retracted into shell. Fig. 21. Larval shell (left side) of the same. Fig. 22. Two-day old veliger. Figs. 23 and 24. *Ianthina prolongata*. Fig. 23. Egg. Fig. 24. Newly hatched veliger. Figs. 25–30. *Natica marochiensis*. Fig. 25. Apex of the adult. Fig. 26. Vertical trans-section of the egg ribbon showing the eggs inside—only part of the section is shown. Fig. 27. Egg capsule showing the egg inside. Fig. 28. Newly hatched veliger. Fig. 29. Larval shell (left side) of the same. Fig. 30. Larval shell (right side) of the same. Figs. 31–35. *Natica tigrina*. Fig. 31. Apex of the adult. Fig. 32. Vertical trans-section of the egg ribbon showing the embryos inside (only part of the section is shown). Fig. 33. Newly hatched veliger. Fig. 34. Same with the animal completely retracted into the shell. Fig. 35. Larval shell (right side) of the same. Figs. 36–40. *Natica* sp. A. Fig. 36. Vertical trans-section of the egg ribbon showing the embryos inside (only part of the section shown). Fig. 37. Egg capsule showing the egg inside. Fig. 38. Newly hatched veliger. Fig. 39. The same with the animal partly retracted into the shell. Fig. 40. Larval shell (right side) of the same.

Text-Figs. 41–59. Figs. 41 & 42. *Natica* sp. B. Fig. 41. Newly hatched veliger. Fig. 42. Larval shell (left side) of the same. Figs. 43–49. *Erronea errones*. Fig. 43. Egg capsule showing the eggs inside. Fig. 44. Egg. Fig. 45. Newly hatched veliger. Fig. 46. The same with the velum and foot partly retracted into the shell. Fig. 47. Larval shell (right side) of the same.

Fig. 48. Sculpture of the larval shell. Fig. 49. Operculum of the larva. Figs. 50–54. *Tonna dolium*. Fig. 50. Apex of the adult. Fig. 51. Egg. Fig. 52. Newly hatched veliger. Fig. 53. Sculpture of the larval shell. Fig. 54. Operculum of the larva. Figs. 55–59. *Tonna* sp. Fig. 55. Egg. Fig. 56. Newly hatched veliger. Fig. 57. Larval shell (right side) of the same. Fig. 58. Larval shell (left side) of the same. Fig. 59. Sculpture of the larval shell.

TEXT-FIGS. 60–81. Figs. 60–69. *Murex virgineus* var. *ponderosa*. Fig. 60. Egg. Fig. 61. Embryo at an early stage. Fig. 62. Embryo of a later stage. Fig. 63. Embryo about to hatch out. Fig. 64. Larval shell (ventral view) of the same. Fig. 65. Larval shell (dorsal view) of the same. Fig. 66. Sculpture of the larval shell. Fig. 67. Veliger, 2-day old and about to settle down. Fig. 68. Veliger just settled down, 2½-day old. Fig. 69. Young one, 20-day old. Figs. 70–78. *Murex trapa*. Fig. 70. Apex of the adult. Fig. 71. Egg capsule. Fig. 72. Egg. Fig. 73. Embryo at the time of hatching. Fig. 74. Larval shell (ventral view) of the same. Fig. 75. Larval shell (dorsal view) of the same. Fig. 76. Young one just settled down after 2½-days of larval life. Fig. 77. Shell of the young one, 2-day old. Fig. 78. Shell of the young one, 12-day old. Figs. 79–81. *Thais bufo*. Fig. 79. Newly hatched veliger. Fig. 80. Same with the velum partly withdrawn into the shell (right side). Fig. 81. Same with the animal nearly retracted into the shell (left side view).

TEXT-FIGS. 82–102. Figs. 82–85. *Thais tissoti*. Fig. 82. Apex of the adult. Fig. 83. Embryo at the time of hatching. Fig. 84. Larval shell (right side) of the same. Fig. 85. Larval shell (ventral view) of the same. Figs. 86–88. *Thais* sp. A. Fig. 86. Embryo about to hatch out. Fig. 87. Larval shell (left side) of the same. Fig. 88. Larval shell (dorsal view) of the same. Fig. 89. *Thais* sp. B. Fig. 89. Newly hatched veliger with the velar lobes nearly retracted into the shell. Figs. 90–92. *Thais* sp. C. Fig. 90. Newly hatched veliger. Fig. 91. Larval shell (left side) of the same. Fig. 92. Larval shell (right side) of the same. Figs. 93–96. *Pyrene versicolor*. Fig. 93. Apex of the adult. Fig. 94. Egg. Fig. 95. Newly hatched veliger. Fig. 96. Larval shell (ventral view) of the same. Figs. 97–104. *Pyrene zebra*. Fig. 97. Apex of the adult. Fig. 98. Egg. Fig. 99. Embryo at the time of hatching. Fig. 100. Veliger, 1-day old. Fig. 101. Same with the animal fully retracted into the shell (right side view). Fig. 102. Two-day old veliger with the velum partly retracted into the shell.

TEXT-FIGS. 103–121. Fig. 103. Larval shell (dorsal view) of the same. Fig. 104. Larval shell (ventral view) of the same. Figs. 105–108. *Pyrene* sp. Fig. 105. Egg. Fig. 106. Newly hatched veliger. Fig. 107. Larval shell (right side) of the same. Fig. 108. Larval shell (left side) of the same. Figs. 109–113. *Nassa jacksoniana*. Fig. 109. Egg. Fig. 110. Embryo at an early stage. Fig. 111. Embryo in the hatching stage. Fig. 112. Newly hatched veliger. Fig. 113. Larval shell (right side) of the same. Figs. 114–119. *Nassa costata*. Fig. 114. Egg. Fig. 115. Embryo just before hatching. Fig. 116. Nelwy hatched veliger. Fig. 117. Veliger, 1-day old. Fig. 118. Seven-day old veliger. Fig. 119. Larval shell of the same. Figs. 120–121. *Nassa thersites*. Fig. 120. Newly hatched veliger. Fig. 121. Same with the animal partly retracted into the shell (left side view).

TEXT-FIGS. 122–146. Figs. 122–125. *Ancilla* sp. Fig. 122. Egg capsule. Fig. 123. Embryo at an early stage. Fig. 124. Embryo at a later stage. Fig. 125. Crawling young one. Figs. 126 and 127. *Xancus rapa*. Fig. 126. Young one, with the animal almost retracted into the shell. Fig. 127. Shell of the young one (dorsal view). Figs. 128–132. *Conus araneosus*. Fig. 128. Egg. Fig. 129. Embryo at an early stage. Fig. 130. Embryo at a later stage. Fig. 131. Embryo at the next stage. Fig. 132. Larval shell of the same. Figs. 133 and 134. *Conus* sp. Fig. 133. Embryo at the time of hatching. Fig. 134. Veliger, 2-day old. Figs. 135–139. Egg capsule A. Fig. 135. Egg. Figs. 136 and 137. Sub-normal embryos at the time of hatching. Fig. 138. Normal embryo at the time of hatching. Fig. 139. Larval shell of the same. Figs. 140–146. Egg capsule B. Fig. 140. Egg. Fig. 141. Embryo 11

days after oviposition. Fig. 142. Embryo 18 days after oviposition. Fig. 143. Embryo about to hatch out. Fig. 144. Larval shell of the same. Fig. 145. Sculpture of the larval shell. Fig. 146. Eight-day old young one.

Explanation of Photographs

Plate XIV

Photo 1. Egg ribbons of *Cerithidea fluviatilis*.

Photo 2. Photomicrograph of a small area of the egg ribbon of *C. fluviatilis* showing the embryos and their surrounding capsules. ca., ×26.

Photo 3. Newly laid egg ribbon of *Cerithium morus*.

Photo 4. Photomicrograph of a small area from one side of the egg ribbon of *C. morus* showing the eggs and their surrounding capsules. ca., ×18.

Photo 5. Egg ribbons (disturbed while dislodging from the substrata) of *Cerithium* sp.

Photo 6. Photomicrograph of a small area of the egg ribbon of *Cerithium* sp. ca., ×24.

Photo 7. Float of *Ianthina prolongata* showing the egg capsules underneath.

Photo 8. Individual egg capsule of *Ianthina prolongata*. Nat. size. 5 mm. in length.

Photo 9. Egg ribbon of *Natica marochiensis*.

Photo 10. Egg ribbon of *Natica tigrina*.

Photo 11. Egg ribbon of *Natica* sp. A.

Photo 12. Egg mass of *Erronea errones*.

Photo 13. Photomicrograph showing the arrangement of the egg capsules in *Erronea errones*. ca., ×8.

Photo 14. Egg ribbon of *Tonna dolium*.

Plate XV

Photo 15. Photomicrograph of a small area of the egg ribbon of *T. dolium* showing the egg capsules and embryos. ca., ×7½.

Photo 16. Egg ribbon (only part of it) of *Tonna* sp.

Photo 17. Photomicrograph of a small area of the egg ribbon of *Tonna* sp. showing the semilunar arrangement of the eggs in each capsule. ca., ×7½.

Photo 18. Same showing the embryos in each capsule. ca, ×6½.

Photo 19. Egg cluster of *Murex virgineus* var. *ponderosa*.

Photo 20. Egg cluster of *Murex trapa*.

Photo 21. Egg cluster of *Thais bufo*.

Photo 22. A few individual capsules of the same.

Photo 23. Egg cluster of *Thais tissoti*.

Photo 24. Two individual capsules (photomicrograph) of the same, showing the embryos inside. ca., ×9½.

Photo 25. Egg cluster of *Thais* sp. A.

Photo 26. Egg cluster of *Thais* sp. B.

PHOTO 27. Egg capsule (photomicrograph) of *Thais* sp. C. Nat. size ca., 3·5 mm. in length.

PHOTO 28. Egg mass of *Pyrene flavida.*

PHOTO 29. Individual capsule (photomicrograph) of the same. Nat. size 2·5 mm. in length.

PHOTO 30. Egg capsule (photomicrograph) of *Pyrene versicolor.* Nat. size. ca., 2 mm.

PHOTO 31. Egg capsules (photomicrograph) of *Pyrene zebra.* Nat. size. ca., 2·2 mm.

PLATE XVI

PHOTO 32. Egg capsules (photomicrograph) of *Pyrene* sp. ca., ×9½

PHOTO 33. Egg capsule (photomicrograph) of *Nassa jacksoniana* showing the eggs inside. ca, ×13½.

PHOTO 34. Part of an egg mass (photomicrograph) of *Nassa costata* showing the embryos inside. ca., ×21.

PHOTO 35. An individual capsule (photomicrograph) of the same showing the egg inside. ca., ×74.

PHOTO 36. Part of an egg mass (photomicrograph) of *Nassa thersites* showing the embryos inside. ca., ×17½.

PHOTO 37. An individual capsule (photomicrograph) of the same showing the egg inside. ca., ×55.

PHOTO 38. Egg capsules of *Ancilla* sp. attached to a polychæte tube.

PHOTO 39. Egg case of *Xancus rapa.*

PHOTO 40. Egg cluster of *Conus araneosus.*

PHOTO 41. Individual capsules of the same.

PHOTO 42. Egg cluster of *Conus* sp.

PHOTO 43. Individual capsule of the same showing the sculpture and exit slit at the top.

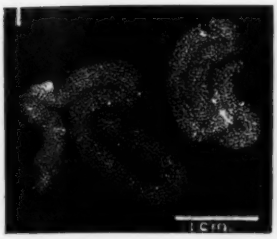
PHOTO 44. Egg cluster of egg capsule A.

PHOTO 45. Individual capsule of the same.

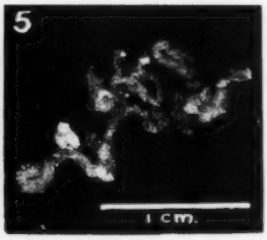
PHOTO 46. Egg cluster of egg capsule B.

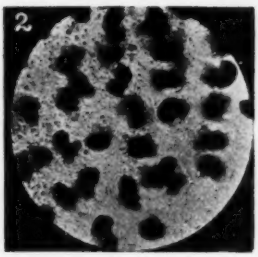
PHOTO 47. Individual capsule of the same.

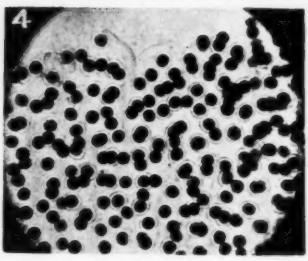
867–57 Printed at The Bangalore Press, Bangalore City, by C. Vasudeva Rao, Superintendent and Published by the Indian Institute of Science, Bangalore-3, India.

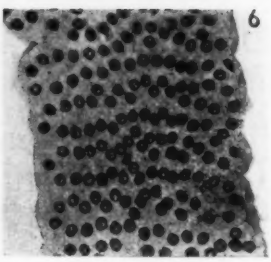

1
1 cm.

3


5
1 cm.

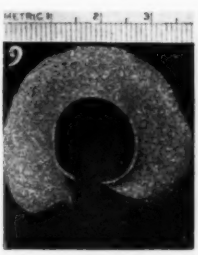

2

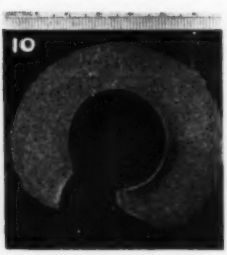

4

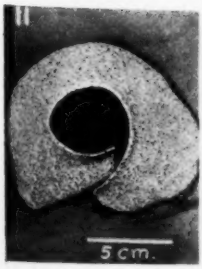

6

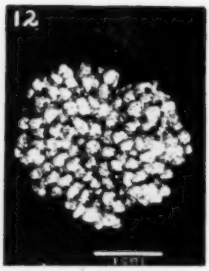
7

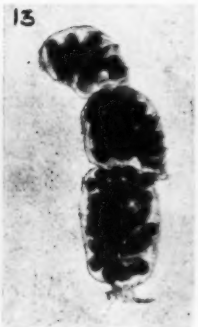
8


9

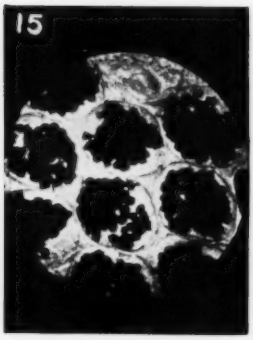

10

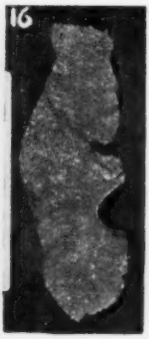

11
5 cm.

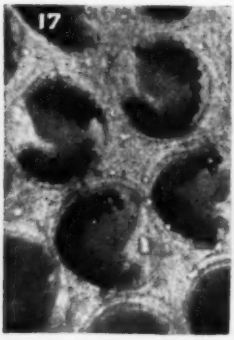

12

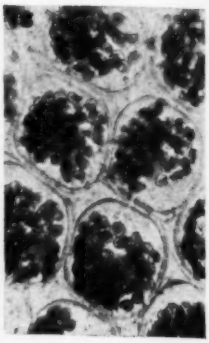

13

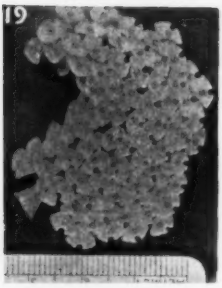
14

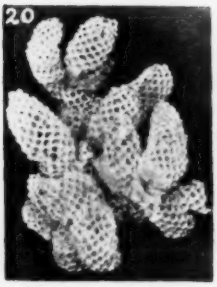

15

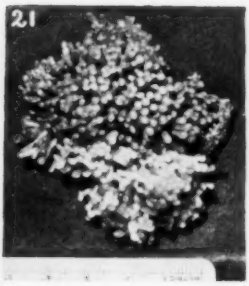

16


17

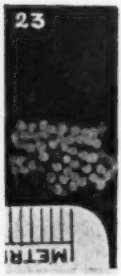



19

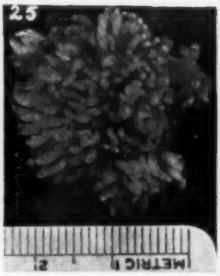

20


21

22
METRIC 1

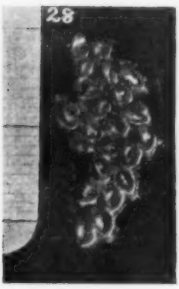

23

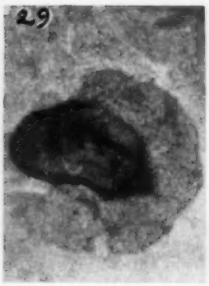
24


25
METRIC 1

26
METRIC 1

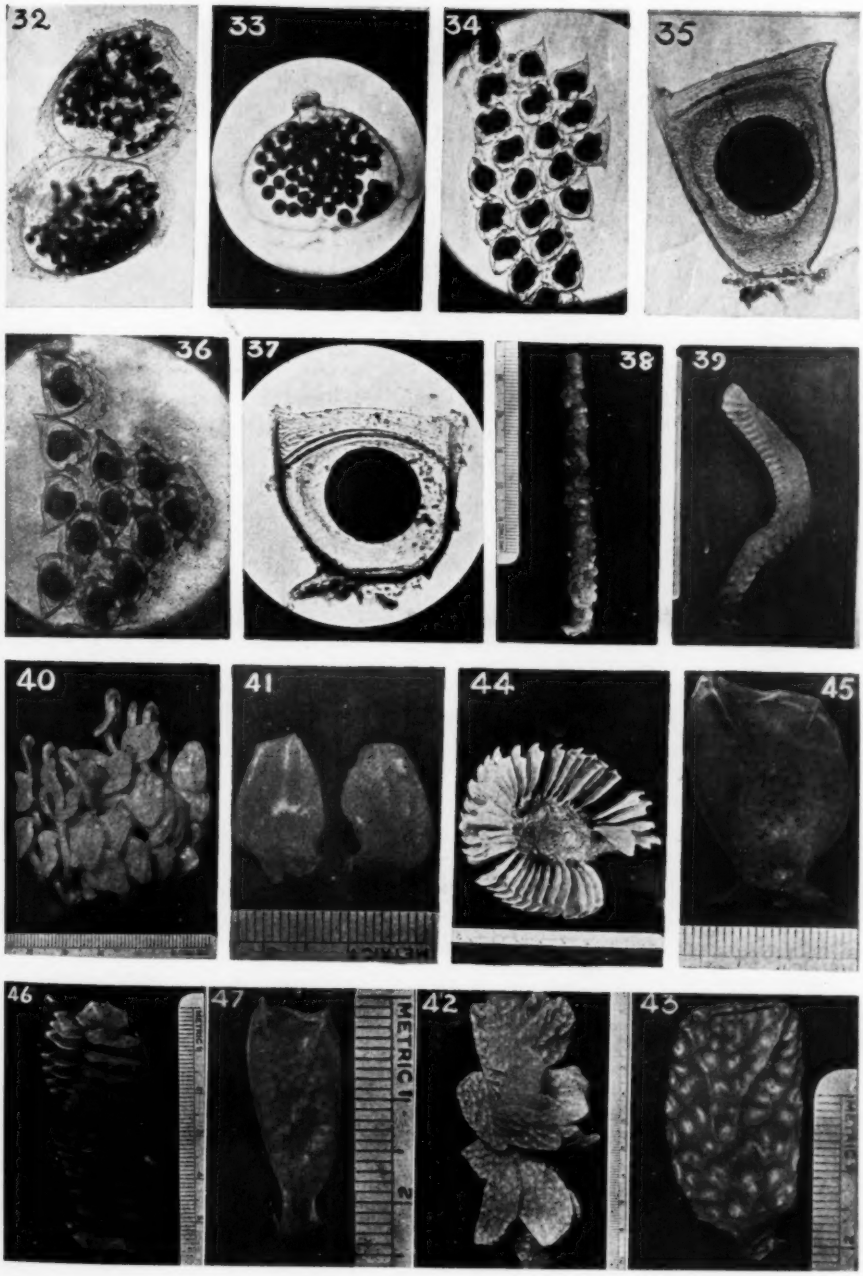
27


28


29

30

31


32
33
34
35
36
37
38
39
40
41
44
45
46
47
42
43
METRIC

THE METABOLISM OF SERINE IN BIOTIN DEFICIENCY

By G. B. Nadkarni and A. Sreenivasan, F.A.Sc.
(Department of Chemical Technology, University of Bombay)

Received May 10, 1957

Serine and ethanolamine occur in phosphatides (Folch and Schneider, 1941). When ethanolamine-1, 2-C^{14} is administered to rat, large amount of radioactivity could be traced in serine of body phosphatides (Pilgeram *et al.*, 1953). The conversion of ethanolamine to serine could be by fixation of carbon dioxide, under appropriate conditions. Such fixation has not been demonstrated, although fixation of $C^{14}O_2$ has been demonstrated in lysine, tyrosine and glutamic acid (Hanke and Siddiqui, 1950; Koppleman *et al.*, 1952) with their corresponding amines.

Biotin has been known to influence CO_2 metabolism (Burke and Winzler, 1943; Shive and Rogers, 1947; Lardy *et al.*, 1947, 1949 *a*, 1949 *b*; McLeod and Lardy, 1949; Feldott and Lardy, 1951; Plaut and Lardy, 1950; Wessman and Werkman, 1950; Ochoa *et al.*, 1947; Lardy and Adler, 1956) and has been shown to influence a number of CO_2 fixation reactions, such as pyruvate to oxalacetate (Shive and Rogers, 1947; Lardy *et al.*, 1947; Wessman and Werkman, 1950; Ochoa *et al.*, 1947), acetone to acetoacetate (Plaut and Lardy, 1950), ornithine to citrulline (Lardy *et al.*, 1949 *a*; Feldott and Lardy, 1951) and propionate to succinate (Lardy and Adler, 1956).

Biotin is also reported to influence the deamination of serine in bacteria (Lichstein and Umbreit, 1947; Lichstein and Christman, 1948). In biotin related deaminases, there is a requirement for biotin-AMP combination (Williams and Christman, 1953). No effect on the deamination rate by *Neurospora* enzyme (Yanofsky, 1953) was obtained with biotin, either alone or in combination with AMP. A function for biotin in the deamination, both oxidative and reductive, of aspartic acid by *Escherichia coli* is inferable from the observed stimulation in the activity of the system when cells of the organism are enriched by incubation with biotin in presence of glucose and inorganic phosphate, preferably after being rendered biotin-deficient by ageing (Gothoskar and Sreenivasan, 1953). The effect of biotin deficiency on deamination has not been studied in the animal organism.

The present work relates to a study of the *in vitro* synthesis of serine from ethanolamine and bicarbonate and of the influence of biotin deficiency on

this system as well as on serine deamination. Serine decarboxylase activity was also followed in view of the reported involvement of biotin in the decarboxylation of succinic (Delwiche, 1950) and oxalacetic (Lichstein and Umbreit, 1947) acids.

EXPERIMENTAL

Young rats (Wistar), initial average weight of 55 g., were kept on a purified biotin-deficient diet (based on Lardy *et al.*, 1949; McCoy *et al.*, 1948) consisting of (percentages): dried (Eakin *et al.*, 1941) raw egg white, 10; alcohol-extracted casein 8; starch 60; sucrose (vitaminised) 10; salt mixture (U.S.P. IV) 4; sesame oil 4; and shark liver oil 2. Vitamin supplements added to the diet were (mg./kg. diet): Thiamine HCl 4; riboflavin 5; niacin 10; calcium pantothenate 20; folic acid 5; pyridoxine HCl 5; vitamin B_{12} 0·2; vitamin K 3; and α-tocopherol 10.

In the diet of the biotin-fed group of rats, egg white was replaced by devitaminised casein and was supplemented with 1·0 mg. biotin per kg. of diet.

The first signs of deficiency such as loss in weight and dermatitis appeared in 8–10 weeks. Animals were then decaptitated and livers quickly removed, chilled and homogenised in cold distilled water to make a 20% suspension.

Ethanolamine to serine conversion.—This system was followed in Warburg flasks with two sidearms similar to the procedure of Lardy *et al.* (1949) and Utter and Kurahashi (1954). Incubations were at 37° C. for half an hour. The main compartment of each flask contained 1·0 ml. of 0·1 M phosphate buffer pH 6·4, 1·0 ml. of 0·2 M ethanolamine solution (pH previously adjusted to 7·0), 0·4 ml. of 0·05 M sodium citrate and 0·1 ml. of 0·05 M sodium fumarate. One sidearm contained 0·5 ml. of 0·1 M sodium bicarbonate and the other contained 0·5 ml. of the liver homogenate. The flasks were equilibrated for 10 minutes at 37° C. before tipping in the contents of both sidearms. Control flasks were kept with the ethanolamine solution replaced by 1·0 ml. of distilled water. The final pH of the reaction mixture was 8·3.

After 30 minutes reaction, 0·5 ml. of 1 N sulphuric acid was added to each flask to stop the reaction. After heating the contents of the flask in a boiling water-bath for 5 minutes, they were centrifuged and an aliquot of the supernatant used for determination of the serine formed microbiologically with either *L. mesenteroides* P-60 (Steele *et al.*, 1949) or *L. casei* (Alexander *et al.*, 1953).

In preliminary experiments, serine formed was detected and determined on paper chromatograms. The method was that of Levine and Chargaff

(1951) with some modifications. The chromatograms were run on paper sheets (Whatman No. 1) which were 45 cm. long, the width depending on the number of spots run. The solutions were spotted at least 3·0 cm. apart. Solutions of serine were spotted alongside by means of standard micropipette. Chromatograms were run by the descending technique, the solvents used being *n*-butanol-acetic acid-water (40 : 10 : 50) and phenol-water (80 : 20). After 18 hours' run, the sheets were dried and the spots developed with 0·4% solution of ninhydrin in acetone. Sometimes the solvent was run twice (Giri and Rao, 1952) and then developed with ninhydrin for clearer separation of the spots. The R_f values for serine with the two solvent systems were 0·18 and 0·40 respectively; those for ethanolamine were 0·33 and 0·75. The spots corresponding to serine were cut out and taken into test-tubes containing 4·0 ml. of 95% ethanol. The contents were carefully shaken for complete extraction. To the extract was added 0·2 ml. of 0·1% copper sulphate when the blue colour was changed to a stable pink. A further 0·8 ml. of ethanol was used to make the volume to 5·0 ml. The density of the colour was read on a Klett-Summerson photoelectric colorimeter using green filter (540 mμ.). The standards for serine were run between 0 and 20 μg. The spots for ethanolamine were fainter as compared to those for serine in equivalent amounts. The values obtained for serine were comparable to those estimated microbiologically.

Serine deamination, serine decarboxylation and liver nitrogen were all determined by methods reported by Nadkarni and Sreenivasan (1957).

Biotin.—Liver homogenate (equivalent to 1 g. of tissue) was hydrolysed with 3% sulphuric acid at 15 lb. pressure for 30 minutes. To the cooled hydrolysate was added 2·0 ml. of 2·5 M sodium acetate and the pH adjusted to neutral with dilute alkali. The volume was made up to 50 ml. Aliquots were used for biotin assay microbiologically with *Lactobacillus arabinosus* (Wright and Skeggs, 1944). The range of biotin concentrations for the standard curve was 0·00005 μg. to 0·00025 μg.

All the values reported are averages for at least quadruplicate independent samples.

Results

From preliminary experiments using paper chromatography for serine assay, it was ascertained that serine was being synthesised from ethanolamine with normal rat liver homogenates to the extent of $1·5 \pm 0·2$ μ moles per mg. N. The values were comparable to those obtained by microbiological assays. In later experiments the extent of serine synthesis from ethanolamine was followed by microbiological assays only.

Table I shows the effect of pH on the synthesis of serine from ethanolamine. Optimum synthesis could take place at pH 8·3.

TABLE I

Effect of pH on ethanolamine to serine conversion

pH of the buffer (0·1 M phosphate buffer)	Resultant pH (after adding bicarbonate)	Serine formed μ moles/mg. N
5·0	6·5	1·18
6·4	8·3	1·64
7·1	9·1	1·27

A decrease in serine formation in biotin deficiency is observable from the results in Table II.

TABLE II

Effect of biotin deficiency on ethanolamine to serine conversion

Group	Average weight gain (10 weeks) (g.)	μ moles of serine per mg. N
− Biotin	+16	0·88±0·04
+ Biotin	+90	1·74±0·04

(Average initial weight of rats was 55 g.)

To some of the biotin-deficient rats, 100 μg. of biotin was administered intraperitoneally, three hours prior to sacrifice. A definite increase in ethanolamine to serine synthesis was observable in these cases. Typical results are given in Table III which also includes data obtained when biotin (10 μg./flask) was added *in vitro* to the system and which shows no stimulation in serine synthesis.

Serine deamination is reduced as a result of biotin deficiency while serine decarboxylation is not affected (Table IV). The effects of biotin deficiency on liver nitrogen and liver biotin are summarised in Table V.

TABLE III

Effect of biotin on ethanolamine to serine conversion

Experiment	Biotin-fed	Biotin deficient (biotin injected)	Biotin deficient (biotin added *in vitro*)
	μ moles of serine per mg. N		
1	1·94	1·73	0·71
2	1·33	1·38	0·88

TABLE IV

Serine deaminase and serine decarboxylase activities

Group	Serine deamination		Serine decarboxylation μl. CO_2/mg.N/30 minutes
	Pyruvic acid	Ammonia	
	μ moles/mg. N		
− Biotin	3·54±0·20	3·47±0·24	3·93±0·20
+ Biotin	5·13±0·20	4·78±0·30	3·71±0·11

TABLE V

Liver analysis

Group	Liver weight per 100 g. body weight (g.)	Liver biotin μg./g. fresh weight	Liver nitrogen	
			Per cent. fresh weight	Per cent. dry weight
− Biotin	5·8±0·4	0·081±0·002	3·33±0·03	11·32±1·0
+ Biotin	2·8±0·3	0·242±0·004	3·89±0·02	13·56±0·8

DISCUSSION

Incorporation of metabolic CO_2 into proteins and amino acids is known (Abelson *et al.*, 1952 *a*, 1952 *b*; Davis *et al.*, 1956). In protein hydrolysates

of micro-organisms, label of $C^{14}O_2$ is contained in the carboxyl group of the amino acids (Abelson *et al.*, 1952 *a*) and this could virtually account for all of the incorporation of radioactivity into the proteins. Fixation of carbon dioxide into amines with formation of corresponding amino acids has also been indicated (Hanke and Siddiqui, 1950; Koppleman *et al.*, 1952).

The intimate relationship between serine and ethanolamine would suggest their interconvertibility, though alternative pathway of ethanolamine to serine is known (Weissbach and Sprinson, 1953). This interconversion is apparently influenced by physiological conditions. The decarboxylation of serine takes place at pH values below 7·0 and is inhibited at higher pH (Nadkarni and Sreenivasan, 1957). The formation of serine from ethanolamine and bicarbonate on the other hand is optimum at pH 8·3 and at lower pH it is less efficient.

The influence of biotin on serine synthesis from ethanolamine is another example of systems wherein CO_2 fixation has been shown to be mediated by the vitamin. Although further work seems necessary, doubts expressed on the coenzymatic role of biotin have recently been answered as due to defects in assay procedures for bound biotin in purified holoenzyme preparations (Lichstein, 1955).

The reduced rate of serine deamination in biotin-deficient animals may be related to the fact that pyruvate metabolism is influenced by this vitamin.

SUMMARY

Synthesis of serine from ethanolamine by carbon dioxide fixation is demonstrated to take place in presence of rat liver enzymes. The system is biotin-dependent.

Serine deaminase activity but not that of serine decarboxylase is also reduced in biotin deficiency.

REFERENCES

Abelson, P. H., Bolton, E. T. and Aldous, E. .. *J. Biol. Chem.*, 1952 *a*, **198**, 165.

———— .. *Ibid.*, 1952 *b*, **198**, 173.

Alexander, J. C., Beckner, W. and Elvehjem, C. A. .. *J. Nutr.*, 1953, **51**, 319.

Burke, D. and Winzler, R. J. .. *Science*, 1943, **97**, 59.

David, J. W., Cheldelin, V. H., Christensen, B. E., and Wang, C. H. .. *Biochim. Biophys. Acta*, 1956, **21**, 101.

Delwiche, E. A. .. *J. Bact.*, 1950, **59**, 439.

Eakin, R. E., Snell, E. E. and Williams, R. J. — *J. Biol. Chem.*, 1941, **140**, 535.

Feldott, G. and Lardy, H. A. .. — *Ibid.*, 1951, **192**, 447.

Folch, J. and Schneider, H. A. .. — *Ibid.*, 1941, **137**, 51.

Giri, K. V. and Rao, N. A. N. .. — *J. Ind. Inst. Sci.*, 1952, **34**, 95.

Gothoskar, S. S. and Sreenivasan, A. — *Ind. J. Med. Res.*, 1953, **41**, 69.

Hanke, M. E. and Siddiqui, M. S. H. — *Fed. Proc.*, 1950, **9**, 181.

Koppleman, R., Mandeles, S. and Hanke, M. E. — *Ibid.*, 1952, **11**, 242.

Lardy, H. A. and Adler, J. .. — *J. Biol. Chem.*, 1956, **219**, 933.

——, McLeod, P. R., Grisolia, S. and Cohen, P. P. — *Ibid.*, 1949 *a*, **180**, 1003.

———, Potter, R. L. and Burris, R. H. — *Ibid.*, 1949 *b*, **179**, 721.

———, ——— and Elvehjem, C. A. — *Ibid.*, 1947, **169**, 451.

Levine, C. and Chargraff, E. .. — *Ibid.*, 1951, **192**, 465.

Lichstein, H. C. .. — *Ibid.*, 1955, **212**, 217.

——— and Christman, J. F. .. — *Ibid.*, 1948, **175**, 649.

——— and Umbreit, W. W. .. — *Ibid.*, 1947, **170**, 423.

McCoy, R. H., McKibben, J. N., Axelrod, A. E. and Hofmann, K. — *Ibid.*, 1948, **176**, 1319.

McLeod, P. R. and Lardy, H. A. .. — *Ibid.*, 1949, **179**, 733.

Nadkarni, G. B. and Sreenivasan, A. — *Proc. Ind. Acad. Sci.*, 1957, **46 B**, 138.

Ochoa, S., Mehler, A., Blanchard, M. L., Jukes, T. H. and Hoffmann, C. E. — *J. Biol. Chem.*, 1947, **170**, 413.

Plaut, G. W. E. and Lardy, H. A. .. — *Ibid.*, 1950, **186**, 705.

Pilgeram, L. O., Gal, E. M., Sassenrath, E. N. and Greenberg, D. M. — *Ibid.*, 1953, **204**, 367.

Shive, W. and Rogers, L. L. .. — *Ibid.*, 1947, **169**, 453.

Steele, B. F., Sauberlich, H. E., Reynolds, M. S. and Baumann, C. A. — *Ibid.*, 1949, **177**, 533.

Utter, M. F. and Kurahashi, K. .. — *Ibid.*, 1954, **207**, 821.

Weissbach, S. and Sprinson, D. B. .. — *Ibid.*, 1953, **203**, 1031.

Wessman, C. E. and Werkman, C. H. — *Arch. Biochem.*, 1950, **26**. 214.

Williams, V. R. and Christman, J. F. — *J. Bact.*, 1953, **65**, 238.

Wright, L. D. and Skeggs, H. R. .. — *Proc. Soc. Expt. Biol. Med.*, 1944, **56**, 95.

Yanofsky, C. .. — *J. Biol. Chem.*, 1953, **202**, 567.

THE METABOLISM OF SERINE IN FOLIC ACID DEFICIENCY

By G. B. Nadkarni and A. Sreenivasan, F.A.Sc.

(Department of Chemical Technology, University of Bombay)

Received May 10, 1957

In a study of choline biosynthesis in the rat, Stekol *et al.* (1952, 1953) indicated that folic acid may be involved in the synthesis of both the ethanolamine and methyl moieties of choline from serine. In earlier work from this laboratory (Fatterpaker, Marfatia and Sreenivasan, 1955), it was observed that the influence of folic acid on choline synthesis may be limited only to the extent to which it serves to provide the ethanolamine moiety of choline. Martin and Beiler (1947) had observed that 3-4-dihydroxy-phenylalanine (DOPA) decarboxylase was reversibly inhibited by folic acid displacing agents such as 7-methyl-folic acid; desoxypyridoxine which is a potent inhibitor of tyrosine decarboxylase was ineffective. It was reported earlier (Nadkarni and Sreenivasan, 1957) that pyridoxine deficiency had no apparent influence on serine decarboxylation. It is now shown that folic acid is involved in serine decarboxylation.

The role of folic acid in glycine-serine interconversion has recently been reported by various workers in the micro-organisms (Holland and Mienke, 1949; Lascelles, Cross and Woods, 1954) and in animals (Totter *et al.*, 1950; Plaut *et al.*, 1950; Elwyn and Sprinson, 1950; Blakley, 1954; Kisliuk and Sakami, 1955; Alexander and Greenberg, 1955; Arnstein, 1955). The work reported here on glycine-serine conversion is discussed in the light of the present available information. Serine deamination was also followed in relation to dietary folic acid.

Experimental

Weanling rats (Wistar) were kept on a purified diet of the following composition (percentages): Alcohol extracted casein 18; starch 60; sucrose (vitaminised) 10; salt mixture (U.S.P. IV) 4; sesame oil 6; shark liver oil 2; and succinyl sulphathiozole 2; vitamin supplements added to the diet (mg. per kg.) were: Thiamine HCl 6; pyridoxine HCl 6; riboflavin 10; calcium pantothenate 20; *p*-aminobenzoic acid 100; nicotinic acid 30; biotin 1·0; vitamin B_{12} 0·5; α-tocopherol 50; vitamin K 10; inositol 500; and choline chloride 500.

One group of animals received the same diet but with 5 mg./kg. diet folic acid added. Folic acid deficiency was inferred from depressed leucocyte count observable after 6–8 weeks. There was also weight loss and loss of hair.

The animals from the deficient and folic acid-fed groups were killed at the end of a 8-week-period by decapitation. Livers were quickly removed, chilled and homogenised in cold distilled water by means of a Potter-Elvehjem homogeniser to a 20% suspension which was used for enzyme studies: Serine decarboxylation, glycine to serine synthesis, serine deamination by methods already outlined (Nadkarni and Sreenivasan, 1957).

Liver folic acid.—Folic acid in autolysed samples was determined microbiologically with *S. fæcalis* R. (Teply and Elvehjem, 1945) using the assay medium of Mitbander and Sreenivasan (1955).

Liver choline.—Total choline in liver and blood and free choline in liver were determined by the method of Appleton *et al.* (1953).

All results are averages of determinations for not less than four different samples.

Results

The effects of folic acid deficiency on leucocyte count and liver levels of folic acid and nitrogen are given in Table I.

Table I

Folic acid deficiency on lecucocyte count and liver levels of folic acid and nitrogen

Group	Liver weight per 100 g. body weight	Leucocyte count	Folic acid in liver μg./g. fresh weight	Liver nitrogen	
				Fresh weight per cent.	Dry weight
−PGA	3·80±0·15	4020±520	2·87±0·03	3·45±0·30	11·01±1·30
+PGA	3·56±0·20	10230±320	5·75±0·04	3·71±0·10	12·84±0·40

In preliminary experiments it was observed that *in vitro* addition of aminopterin inhibited serine decarboxylation. Thus the activity (μl. CO_2/mg. N/30 minutes) in presence of 200 μg. of aminopterin was 1·60 as compared to 3·95 without added aminopterin. To ascertain if aminopterin inhibition is observable *in vivo*, rats (120 g. wt.) were given 25 μg. per day aminopterin intraperitoneally for 6 days. They were then sacrificed. The

results obtained for serine decarboxylase activity (μl. CO_2/mg. N/30 minutes) were $1 \cdot 89 \pm 0 \cdot 08$ as compared to $3 \cdot 61 \pm 0 \cdot 03$ for the control animals.

Dietary deficiency of folic acid also depressed serine decarboxylase activity (Table II). Glycine to serine conversion was also reduced in the deficient animals. Serine deamination was unaffected.

TABLE II

Folic acid deficiency and serine decarboxylation, glycine to serine synthesis and serine deamination

Group	Serine decarboxylation QCO_2 μl. CO_2/mg. N/30 minutes	Glycine to serine conversion μ moles of serine per mg. N	Serine deamination μ moles of NH_3 per mg. N
– PGA	$2 \cdot 52 \pm 0 \cdot 10$	$0 \cdot 72 \pm 0 \cdot 14$	$3 \cdot 67 \pm 0 \cdot 10$
+ PGA	$3 \cdot 68 \pm 0 \cdot 05$	$1 \cdot 36 \pm 0 \cdot 12$	$3 \cdot 89 \pm 0 \cdot 02$

The effects *in vitro* and *in vivo* of folic acid on serine decarboxylation and on glycine to serine conversion are shown in Table III. Folic acid-deficient rats were used in all these experiments. Folic acid was given (200 μg. per rat) intraperitoneally 24 hours prior to sacrifice. *In vitro* folic acid was added to the system in a final concentration of 60 μg. per ml.

TABLE III

Folic acid and serine decarboxylation and glycine to serine conversion

System	*In vitro*		*In vivo* 200 μg. PGA/rat
	No addition	60 μg./ml. PGA	
	μl. CO_2 per mg. N per 30 minutes		
Serine decarboxylation	2·74	3·68	3·94
	μ moles of serine per mg. N		
Glycine to serine synthesis	0·68	0·71	1·14

Folic acid administration *in vivo* nearly restores the impairment in the activities of the two systems in folic acid deficiency. *In vitro* effect is observable to some extent in serine decarboxylation.

Choline contents of blood and liver are shown in Table IV. It may be seen that in folic acid deficiency the levels for free and total choline of liver as well as for blood choline are less. An influence of the vitamin on phospholipid levels of liver was reported by Fatterpaker, Marfatia and Sreenivasan (1955 *e*).

TABLE IV

Choline levels of blood and liver

Group	Liver choline		Blood choline (Total)
	Free	Total	
	mg. per g. fresh weight		mg. per ml.
− PGA	0·183±0·007	5·34±0·04	0·624±0·021
+ PGA	0·243±0·003	6·10±0·02	0·816±0·030

DISCUSSION

Earlier work from this laboratory (Fatterpaker, Marfatia and Sreenivasan, 1955) had suggested that the influence of folic acid on methyl metabolism may be confined only to the formation of the ethanolamine moiety of choline. The present observations have shown more directly that folic acid aids in the conversion of serine to ethanolamine.

Stetten (1941) and Arnstein (1951) had shown that glycine serves as a precursor of the ethanolamine moiety of choline. Glycine is first converted to serine which is then decarboxylated to ethanolamine. The involvement of folic acid in the conversion of glycine to serine has been variously reported (*vide infra*) and is confirmed by the present studies. However, the vitamin is also concerned in the further metabolism of serine to ethanolamine.

The function of folic acid in the biosynthesis of ethanolamine is further borne out by the data on choline levels of liver and blood. The somewhat more marked lowering of free choline as compared to the bound choline would suggest a more direct influence of the vitamin on newly formed choline.

SUMMARY

Aminopterin inhibits serine decarboxylation in rat liver homogenates.

Dietary deficiency of folic acid in rats also reduces serine decarboxylase activity.

Folic acid does not influence serine deamination, but glycine to serine synthesis is impaired in a dietary deficiency of the vitamin.

In folic acid deficiency liver and blood levels of choline are also lowered.

REFERENCES

Alexander, N. and Greenberg, D. M. *J. Biol. Chem.*, 1955, **214**, 821.

Appleton, H. D., LaDu, B. N., Levy, B. B., Steele, J. M. and Brodie, B. B. *Ibid.*, 1953, **205**, 803.

Arnstein, H. R. V. .. *Biochem. J.*, 1951, **48**, 27.

——— .. *Ibid.*, 1955, **60**, vii.

Blakley, R. L. .. *Ibid.*, 1954, **58**, 448.

Elwyn, D. and Sprinson, D. B. .. *J. Biol. Chem.*, 1950, **184**, 475.

Fatterpaker, P., Marfatia, U. and Sreenivasan, A. *Ind. Jour. Med. Res.*, 1955 *a*, **43**, 43. (*Nature*, 1951, **167**, 1067.)

———, *Ibid.*, 1955 *b*, **43**, 343.
(*Ibid.*, 1952 *a*, **169**, 1096).

———, *Ibid.*, 1955 *c*, **43**, 337.
(*Ibid.*, 1952 *b*, **170**, 894).

———, *Ibid.*, 1955 *d*, **43**, 349.
(*Ibid.*, 1954, **173**, 359).

———, *Biochem. J.*, 1955 *e*, **59**, 470.

Holland, B. R. and Mienke, W. W. *J. Biol. Chem.*, 1949, **178**, 7.

Kisliuk, R. L. and Sakami, W. .. *Ibid.*, 1955, **214**, 47.

Lascelles, J., Cross, M. J. and Woods, D. D. *J. Gen. Microbiol.*, 1954, **10**, 267.

Martin, G. J. and Beiler, J. M. .. *Arch. Biochem.*, 1947, **15**, 201.

Mitbander, V. B. and Sreenivasan, A. *Arch. fur. Mikrobiologie*, 1955, **21**, 60.

Nadkarni, G. B. and Sreenivasan, A. *Proc. Ind. Acad. Sci.*, 1957, **46 B**, 138.

Plaut, G. W. E., Betheil, J. J. and Lardy, H. A. *J. Biol. Chem.*, 1950, **184**, 795.

Stekol, J. A., Weiss, K. and Weiss, S. *Arch. Biochem. Biophys.*, 1952, **36**, 5.

———, ———, Smith, P. and Weiss, S. *J. Biol. Chem.*, 1953, **201**, 299.

Stetten, D., Jr. .. *Ibid.*, 1941, **140**, 143.

Teply, L. J. and Elvehjem, C. A. .. *Ibid.*, 1945, **157**, 303.

Totter, J. R., Kelley, B., Day, P. L. and Edwards, R. R. *Ibid.*, 1950, **186**, 145.

HYMENIA RECURVALIS F. AND ITS PARASITE COMPLEX

By E. S. Narayanan, F.A.Sc., B. R. Subba Rao and M. Ramachandra Rao
(*Division of Entomology, Indian Agricultural Research Institute, New Delhi*)

Received May 13, 1957

Introduction

Hymenia recurvalis F. is one of the sporadic pests of irrigated vegetable crops, especially the leafy vegetables. It is known to have an almost world-wide distribution and is of common occurrence in our country during the warmer months both in the hills and plains all over India. It webs the leaf, feeds on the green matter and pupates within the rolled leaf. It is found to attack most of the leafy vegetables, the main host plant being *Amaranthus* sp. and is also known to attack maize, beet root and sweet potato. In this paper observations made in the field as well as in the laboratory with regard to the host parasite relationship of *Hymenia recurvalis* and its parasites have been explained and discussed. No sampling methods have been undertaken to calculate the percentage of damage caused by the pest or the population of the pest per unit area, but as trained observers in the field the authors could estimate the abundance of the host in the field and the damage caused by the pest.

Observations

During the months of July and August 1956, the *Amaranthus* crop in the vegetable gardens of the Indian Agricultural Research Institute was found to have been severely attacked by *Hymenia recurvalis*. Almost every plant was infested with a number of larvæ and it could be well said that the insect was present in an epidemic form. At the same time a number of cocoons of the parasite *Apanteles* new species were also seen on the lower surface of the leaves. Laboratory rearing of the host larvæ collected from the field revealed that the pest was attacked by three primary parasites of potential importance; the most abundant being the *Apanteles* new species, the lesser being an unidentified Ichneumonid and a Braconid. Very few pupæ of the latter two species could be collected from the field nor could be reared from the host larvæ collected from the field. But in the new *Apanteles* sp. the case was different. Collections of pupæ of *Apanteles* and also laboratory rearing of the host larvæ collected from the field revealed that the parasitisation by *Apanteles* was observed to be as high as 60% and in every case only

one parasite emerged from each host larva. This high population of the species remained throughout the months of July, August and also a part of September as evidenced by the laboratory rearing of the host as well as from the pupæ of the parasite collected from the field. Surprisingly the host population in the field also remained more or less stable throughout the period under observation in spite of the fact that it was being attacked by three primary parasites and also being subjected to a high degree of parasitisation by *Apanteles* sp. In order to investigate the causes for the abundance of the host population in spite of the presence of its parasites, careful field and laboratory investigations were undertaken. *Apanteles* pupæ were collected from the field at frequent intervals to find out in the laboratory the percentage of actual emergence of the adult parasites from the pupæ. The *Apanteles* pupæ collected during the months of July and August were mainly found to be hyperparasitised by an Elasmid (*Elasmus* sp.), an Eurytomid (*Eurytoma* sp.), a Ceraphronid (*Ceraphron* sp.), and those pupæ collected in the month of September were mainly hyperparasitised by the Elasmid and two species of Eulophid (*Entedon* sp. and *Mestocharis* sp.) and a species of Pteromalid to a lesser extent.

Two interesting points revealed by this phenomenon of hyperparasitism as evidenced by the following tables are that (1) the degree of hyperparasitism varied greatly between the different secondary parasites attacking the *Apanteles* pupæ at the same time, (2) that there was more or less a sort of seasonal distribution and abundance of these hyperparasites that made their appearance to constitute this parasite complex. For example, among the five different hyperparasites that attacked the *Apanteles* pupæ in the month of July, the degree of hyperparasitism was very high in the case of the Elasmid and Ceraphronid in comparison with the rest of the three species. Similarly with regard to the tendency towards the seasonal prevalence of the hyperparasites, laboratory studies and observations revealed that only the Elasmid and Ceraphronid were dominantly present in the month of July, whereas the Eupelmid appeared only at a later stage, namely in the month of August. The pupæ collected in the month of September were attacked by two different species of Eulophids which had not hitherto made their appearance, as will be seen in the following table and graphically represented in Fig. 1.

DISCUSSION

Observations in the field disclosed that the population of the pest was constantly abundant throughout the period under study, that is, till the middle of the month of September in spite of the fact that the host was being attacked by three primary parasites and that there was altogether an overall high degree

of parasitisation. By the middle of September the host was observed to have completed two broods and was running the third generation whereas by the same time *Apanteles* new species should have reached its fifth generation since its life-cycle ranged only from 15–20 days as compared to the long

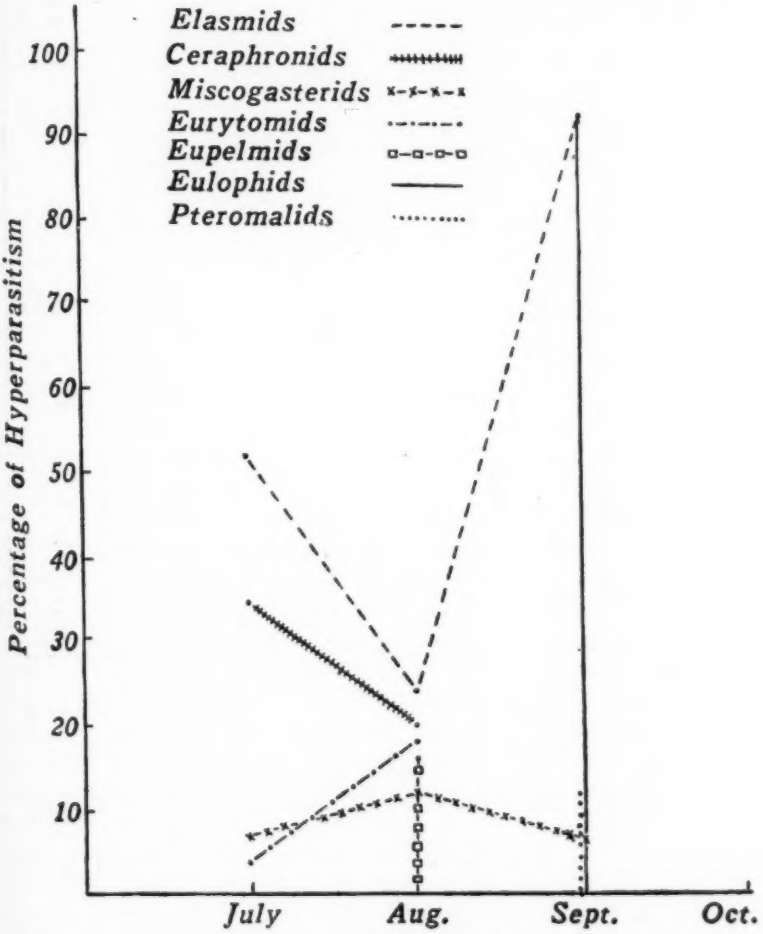


FIG. 1. The Percentage and Sequence of Hyperparasites on *Hymenia recurvalis* F.

life-cycle of the host. Notwithstanding the fact that it was a solitary parasite it should have brought down the host population at least to a considerable extent mainly on the strength of its being ahead of the host by two generations ccording to Thompson (1922) as quoted by Sweetman (1936). According

TABLE I

The percentage and sequence of primary parasites on Hymenia recurvalis *F.*

Month	No. of Hymenia larvæ collected from field and reared	No. of *Apanteles* adults			No. of Ichneumonids			No. of Braconids		
		Reared in the laboratory from the larvæ collected from the field	Pupæ collected from the field	Percentage of parasitism	Reared from the host larvæ collected from the field	Pupæ collected from the field	Percentage of parasitism	Reared in the laboratory from the field collected larvæ	Pupæ collected from the field	percentage of parasitism
July ..	22	13	67	..	1	1	..	1	1	..
August ..	42	26	34	..	2	1	..	2	2	..
September ..	23	14	208	..	1	..	..	..	..	..
Total ..	87	53	309	61%	4	2	4·6%	3	3	3·4%

TABLE II

The percentage of hyperparasitism on Apanteles *new species and the degree of hyperparasitism among various hyperparasites and their seasonal occurrence*

Month	No. of *Apanteles* pupæ collected from the field	No. of pupæ hyperparasitised	Total percentage hyperparasitised	No. of pupæ of *Apanteles* hyperparasitised by						
				Elasmids	Cera-phronids	Miscogas-terids	Eurytomids	Eupelmids	Eulophids	Pteromalids
July ..	67	42	..	22	15	3	2	Nil	Nil	Nil
August ..	34	25	..	6	5	3	7	4	Nil	Nil
September ..	208	130	..	62	Nil	4	Nil	Nil	57	7
Total ..	309	197	63·7	90	20	10	9	4	57	7

to him if the reproductive potential of a parasite exceeds the reproductive potential of the host, then the time which will elapse before the parasite theoretically will equal the host in numbers and so exterminate it, can be determined from the formula

$$t = \frac{\log\left(\frac{na - n - pa}{pa}\right)}{\log a}$$

where

n = the initial number of hosts,

p = the initial number of parasites,

t = the required time, expressed in number of parasite generations, and

$$a = \frac{\text{the reproductive potential of the parasite}}{\text{the reproductive potential of the host}}.$$

However, the high percentage of parasitisation by the *Apanteles* sp. in association with the two other primary parasites could not bring down the population of the host. The failure of natural control by the parasites here is mainly due to the presence of hyperparasites that attacked *Apanteles* pupæ and may not be due to the high reproductive potential of the host or the unequal sex ratio among the host and the parasites even though it is possible they may have played a role but certainly only a very lesser role. Hyperparasitism as evidenced here was a significant factor in the maintenance of the balance between the insect species involved. Here is a classic example of the biological association of the host, primary parasites and secondary parasites forming an amazingly well-adjusted complex. The hyperparasites certainly delayed considerably the time when the primary parasite (*Apanteles* sp.) would become sufficiently abundant to check the host population appreciably. As for the phenomenon that a large number of hyperparasites of different families attacked the *Apanteles* pupæ can be explained by the continued abundance of these parasites in the locality, the non-availability in sufficient numbers of their preferred host species and the subsequent adaptability to the host available in plenty rather than to the laws of specificity and host selection. The variation in the degree of hyperparasitism among the various secondary parasites involved may be ascribed to the high reproductive potential of the available secondary parasites. It was also remarkable that from each pupa only one particular species of hyperparasite emerged. In other words, there was no multiparasitism in this remarkable phase of

hyperparasitism. Could it be that this occurrence is only a way to keep the balance of population in nature?

SUMMARY

1. *Hymenia recurvalis* F. was found to be attacked by three primary parasites namely (1) *Apanteles* new species, (2) an unidentified Ichneumonid and (3) an unidentified Braconid.

2. The degree of parasitism was observed to be about 62% in case of *Apanteles* sp. and not significant in the two other species.

3. In spite of the two observations cited above, the pest was found to be abundant in the field throughout the period under observation and the population of *Apantales* sp. also remained more or less constant.

4. Investigations into the failure of the natural control by parasites to check the host population revealed the presence of eight different species of hyperparasites attacking the pupæ of *Apanteles* sp. and constituted a major factor in the maintenance of balance between the host and the parasites.

5. There is a wide field for research in this direction which will attract some of the younger and energetic entomologists of our country.

ACKNOWLEDGEMENT

We are grateful to Dr. B. P. Pal, Director, Indian Agricultural Research Institute, New Delhi, for his keen interest in this piece of research.

REFERENCE

Sweetman, H. L. .. *The Biological Control of Insects*, Comstock Publishing Co., New York, 1936.

CONTRIBUTION TO OUR KNOWLEDGE OF THE PHYSIOLOGICAL ANATOMY OF SOME INDIAN HYDROPHYTES

VI. The Stem of *Hygrophila quadrivalvis* Nees

By M. V. Mirashi, M.Sc.

(*Department of Botany, College of Science, Nagpur*)

Received May 6, 1957

(Communicated by Dr. T. S. Sabnis, F.A.Sc.)

Introduction

Hygrophila R.Br. is a typical hydrophytic genus of the Acanthaceæ. In India, it is represented by seven species which grow in marshy areas. *H. quadrivalvis* Nees is a rare species of this genus. It is an erect, stout, hairy* annual herb which grows to a height of about two and half feet. "It helps to make the perfect transitional series from the smaller and simpler Hygrophilas to *Asteracantha*" (Santapau, 1951).

The plants come up by the end of August. The flowering season extends from October to December. Some plants, however, come up late in the season. These can be observed to continue even after the substratum has fairly dried up.

The stem of this species is quadrangular in outline and often red in colour. It is profusely hairy, especially in the younger parts. It is erect or slightly decumbent near the base where it roots at the nodes.

Material and Method

Hygrophila quadrivalvis Nees is restricted to a very small area near the source of the Nag River in Nagpur. It occurs in thick dense stands in the puddles where the water is almost stagnant. Collections were made at different periods during the year and the anatomical characters were studied mostly

* The species is classed as "glabrous or nearly so" by Haines, although in the detailed notes on the species, he describes it as a "rather coarse herb with strigillose or glabrescent stem and leaves which are hairy above". "Glabrate" is the word used for the leaves of this plant by C. B. Clarke in Hooker, *f. Fl. Brit. India*, **4**, 408. The word is not found in the original descriptions of the species by Nees. From these descriptions, however, it appears that there are various forms of this plant. The Nagpur plants have profusely hairy stems as well as leaves which are hairy on both surfaces. This striking hairiness is in accordance with Nees's own remarks. The author is indebted to Father H. Santapau, St. Xavier's College, Bombay, for this information about the species.

from free-hand sections of fresh as well as preserved material. Considerable difficulty was experienced in sectioning the material on account of the abundant occurrence of cystoliths in the epidermis of the stem.

DESCRIPTION

1. *Epidermis.*—Outer walls thickened, radial walls thin and undulating; cuticle thin, even in specimens collected quite late in the season (Figs. 6 and 7). Solitary elongated cystoliths abundant in large epidermal cells (Figs. 4 and 5). Stomata situated in groups, caryophyllaceous, raised well above the general level of the epidermal cells (Fig. 1). Multicellular, peltate glands frequent (Fig. 2). Multicellular, uniseriate hairs with bulbous bases (Fig. 3).

Epidermal cells of the lower portion of the stem, which is under water or embedded in the saturated soil, larger in size, with the outer walls and cuticle thinner than in the upper aerial portion (Figs. 6 and 7).

2. *Cortex.*—As in the stems of several species of the Acanthaceæ, the outer cortex is mostly collenchymatous, being interrupted at places by radial sectors of chlorenchyma (Fig. 6). Collenchymatous thickening less pronounced and the cells tangentially elongated in the lower portion of the stem (Fig. 7).

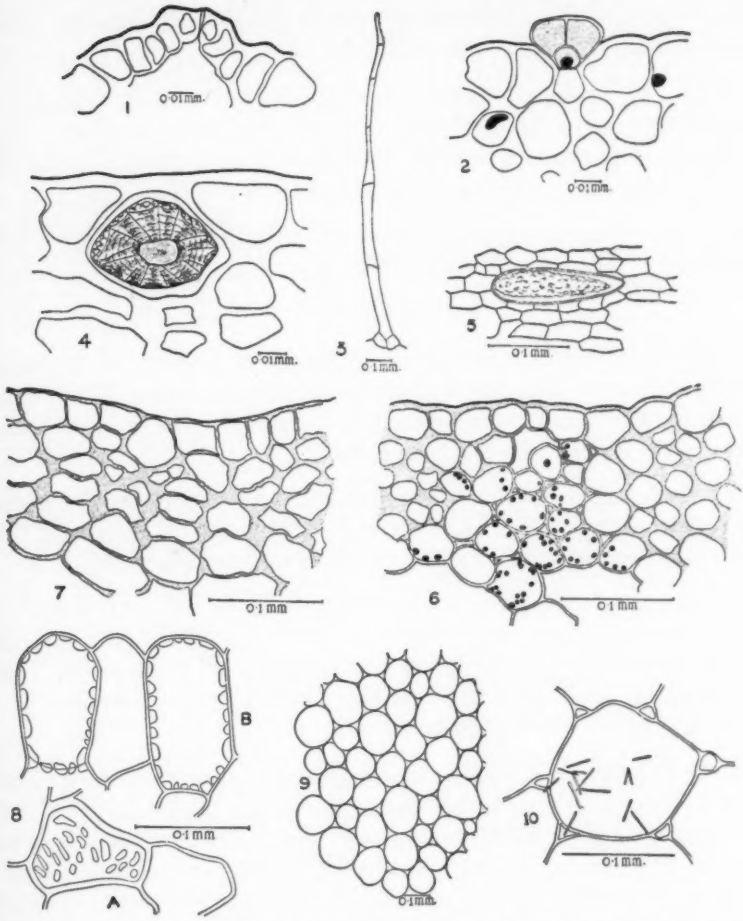
Middle cortex consists of thin-walled chlorophyllose cells separated by schizogenous air-spaces. The cortical cells abound in raphides. In the upper portion of the stem the cells are roundish and the lacunæ are smaller. The cells of the lacunar cortex in the lower portion hypertrophy, stretch in various directions and remain attached to each other by their ends only. The lacunæ enlarge and become radially elongated. Thus, the cortex in the lower portion is a broad zone forming a spongy covering for the stem.

Sections of the material collected later in the season reveal a gradual narrowing of the lacunar cortex. There is a deposition of tannin in the cortical cells. Ultimately, along with the peripheral collenchyma, the lacunar middle cortex gets crushed so that in the older stem even the outlines of the constituent cells cannot be made out (Figs. 13 and 14).

Some of the cortical cells develop, lignified and pitted plates on their walls late in the season (Figs. 8 and 13).

The cells of the inner cortex show the presence of anthocyanin. The endodermis is distinct. While the outer and middle cortex are getting crushed, this layer is seen to become meristematic; but, it does not give rise to any secondary tissue (Fig. 14).

A subepidermal phellogen gives rise to lenticels (Fig. 15).



TEXT-FIGS. 1–10. *Hygrophila quadrivalvis* Nees.—Fig. 1. Part of the T.S. of the stem showing the raised stoma. Fig. 2. Part of the T.S. of the stem showing the multicellular peltate gland in the epidermis. Fig. 3. The multicellular, uniseriate hair. Fig. 4. Part of the T.S. of the stem showing the cystolith in the large-sized epidermal cell. Fig. 5. Part of the epidermal peel showing the elongated solitary cystolith. Fig. 6. Part of the T.S. of the upper aerial part of the stem showing the well-developed collenchyma of the outer cortex interrupted by the radial sector of thin-walled chlorenchyma. Fig. 7. Part of the T.S. of the lower submerged portion of the stem showing the tangential elongation of the epidermis and the outer cortical cells as well as the comparatively poorer collenchymatous thickening. Fig. 8. (A) Part of the T.S. of the stem showing the supporting cell in the cortex with the lignified pitted plate in surface view; (B) Part of the longitudinal section of the stem showing two supporting cells with the characteristic pitted thickening on their walls. Fig. 9. Part of the T.S. of the stem showing the thin-walled parenchymatous pith with very small intercellular spaces. Fig. 10. Part of the T.S. of the stem showing the raphides in the pith cell.

4. *Pericycle*.—This region is not clearly marked out from the phloem. It includes several strongly thickened fibres.

5. *Vascular system*.—This consists of separate vascular bundles that get joined together by the formation of the interfascicular cambium. Secondary growth in thickness is normal. Phloem includes a number of bast-fibres (Fig. 16). There is a well-developed secondary wood (Fig. 17).

6. *Pith*.—Thin-walled parenchymatous cells; intercellular spaces very small (Fig. 9). Raphides abundant (Fig. 10).

DISCUSSION

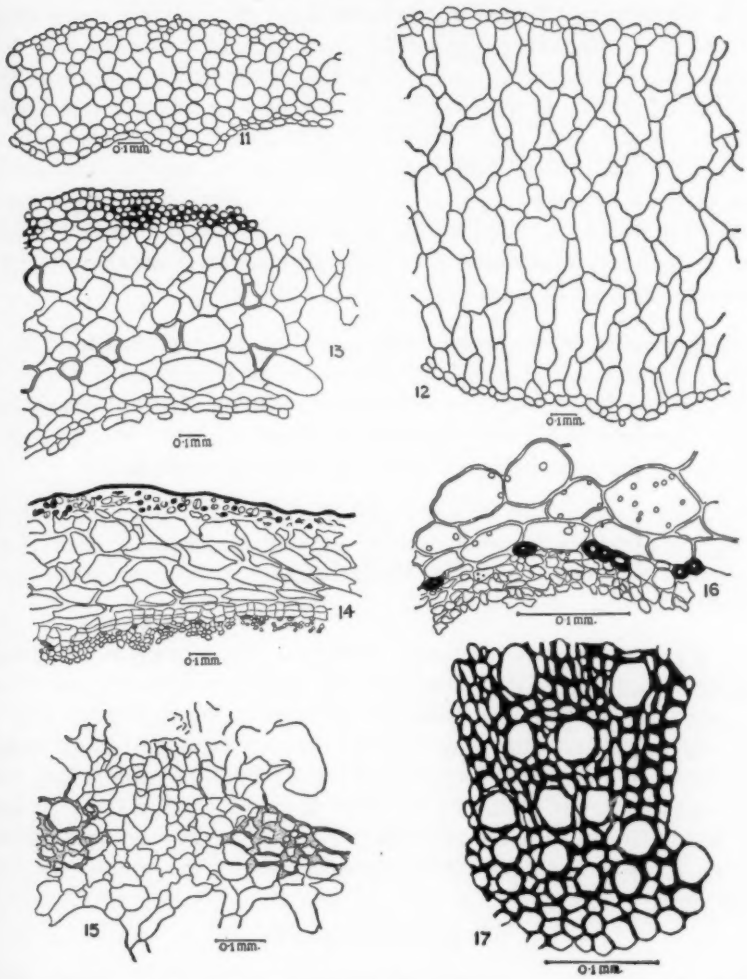
1. The foregoing account of the physiological anatomy of the stem of *Hygrophila quadrivalvis* Nees confirms the amphibious nature of the species. It reveals the characteristic combination of hydrophytic and geophytic characters met with typically in the helophytes.

The thick-walled epidermis, the profuse covering of hairs, the presence of the cuticle, the collenchymatous outer cortex and the well-developed xylem elements indicate essentially an adaptation to terrestrial conditions.

The peripheral mechanical cylinder comprising the thick-walled epidermis and the collenchymatous outer cortex, as well as the broad woody cylinder of secondary xylem, situated round the periphery of a prominent central pith, give to the stem mechanical rigidity which is further enhanced by the presence of pericyclic and bast-fibres.

2. The fragile lacunar cortex is strengthened not only by the peripheral shell of collenchyma, but also by the thick-walled, lignified and pitted cells. These are somewhat prosenchymatous and occur singly or in groups of twos or threes. They develop rather late in the season by secondary sclerosis of thin-walled cells. The distribution of these cells and the lignified thickening of their walls suggest that they serve as local mechanical supports. Such an arrangement for supporting the lacunar cortical tissue of the hydrophytic stem is not very frequently met with. It has been recorded earlier by the author (1957) in the stem of *Lobelia alsinoides* Lamk. in which the cells are, however, not lignified. Isolated supporting cells with peculiar thickening ridges have been recorded by Mullan (1932) in the pneumatophore of the Indian halophytes, *Avicennia alba* Bl. and *A. officinalis* Linn.

3. As is characteristic of the stems of marsh plants, the conducting system of this stem is well developed in the form of a broad zone of wood and the correspondingly elaborate phloem.



TEXT FIGS. 11–17. *Hygrophila quadrivalvis* Nees.—Fig. 11. Part of the T.S. of the upper aerial portion of the stem showing the thin-walled spherical cells and the rather small intercellular spaces of the middle cortex. Fig. 12. Part of the T.S. of the lower submerged portion of the stem showing the hypertrophied cells of the middle cortex and the radially elongated intercellular spaces. Fig. 13. Part of the T.S. of the stem, collected rather late in the season, showing the narrowing of the middle cortex and the development of supporting cells. Fig. 14. Part of the T.S. of the stem, collected still later, showing the lacunar middle cortex getting crushed so that the outlines of the constituent cells can hardly be made out. Note the secondary meristem. Fig. 15. Part of the T.S. of the stem showing the lenticel. Fig. 16. Part of the T.S. of the stem showing the endodermis, the pericycle, that includes some sclerenchymatous fibres, and the phloem tissues. Fig. 17. Part of the T.S. of the stem showing the well-developed wood elements.

4. The primary lacunar cortex constitutes the aerating tissue of the stem. As in the stems of the hydrophytic members of the Compositæ, Scrophulariaceæ and Lobeliaceæ, described earlier by the author, the upper aerial portion of this stem and its lower part, which may be submerged or situated in poorly aerated soil, show significant differences in their anatomy. In the latter, the walls of the epidermal cells are thinner and the collenchyma of the outer cortex shows poorer development. The aquatic environment also seems to act as a stimulus for the tangential expansion of these peripheral tissues so that they could keep pace with the elaborated aerating system of the middle cortex in the lower portion of the stem.

The expansion of the cortex, consequent on the enlargement of the air chambers and the elongation of the thin-walled parenchymatous cells of the middle cortex, brings about a more central disposition of the mechanical elements of the wood in the lower portion of the stem which has got to be inextensible rather than inflexible, unlike the aerial counterpart.

A similar development of a profusely lacunar cortex has been described by Solereder (1908) in the allied species, *Astercantha longifolia* Nees (*Hygrophila spinosa* T. Anders.), and by Mullan (1932) in the stem of the Indian halophyte, *Acanthus ilicifolius* Linn.

5. With the improved aeration of the soil, consequent on its gradual drying up with march of the season, the secondary wood shows a rapid and pronounced development, the need for aeration is less keenly felt and the lacunar cortex goes on narrowing till it is ultimately crushed. These changes are significant in view of the fact, pointed out earlier by Saxton (1924), while explaining his theory of " mixed formations in time ", that in India the same species has to live often under two different sets of environmental conditions during its life-time and that the responses shown by the plants to these are striking.

ACKNOWLEDGEMENT

The author wishes to express his gratitude to Dr. T. S. Sabnis, D.SC., F.A.SC., for kindly going through the manuscript of this paper and making valuable suggestions.

LITERATURE CITED

Mirashi, M. V. .. "Contribution to our knowledge of the physiological anatomy of some Indian Hydrophtes, VI. The stem of *Lobelia alsinoides* Lam.," *Proc. Ind. Acad. Sci.*, 1957, **45** (4), 181-185.

Mullan, D. P. .. "Observations on the biology and physiological anatomy of some Indian Halophytes," *J. Indian bot. Soc.*, 1932, **11**, 285–302.

Santapau, H. .. *The Acanthaceæ of Bombay*, University of Bombay Botanical Memoirs, No. 2, Bombay, 1951.

Saxton, W. T. .. "Phases of vegetation under monsoon conditions," *J. Ecol.*, 1924, **12**, 1–38.

Solereder, H. .. *Systematic Anatomy of the Dicotyledons* (English Translation), Oxford, 1908.

SOME OBSERVATIONS ON THE REPRODUCTION OF THE MILKFISH *CHANOS CHANOS* (FORSKÅL)

By P. R. S. Tampi

(*Central Marine Fisheries Research Station, Mandapam*)

Received April 20, 1957

(Communicated by Dr. N. K. Panikkar, F.A.SC.)

Introduction

Several aspects of reproduction of the milkfish are still baffling and the progressive reproductive phases of the gonads and the spawning cycle in the fish are not properly understood. The validity of a statement by Chaudhuri (1916) that the ripe roe of *Chanos* is dried, smoked and sold by fishermen around the Chilka Lake which conveys an impression of common occurrence of the breeders was inquired into and it is understood that the fish are now rare in these places. Apart from the occasional notes on the well developed roe and the number of eggs produced by the fish, by Sunier (1922) and Adams *et al.* (1932), the only critical approach to the problem has been by Bunag (1951) who made an effort to ascertain the spawning habits of the fish on the basis of ova diameter measurements of the developing ovarian eggs. This extreme paucity of literature is largely owing to the difficulty in obtaining sufficient material for study, particularly of the more advanced stages in the development of the gonads. For the same reason, although rarely mature gonads have been seen in a few fish caught from the sea, it is rather hard to build up a connected picture of the developing reproductive organs; the result has been that no reliable account of the frequency of spawning or size at maturity are available. The condition of gonads in the majority of specimens caught from the sea in the fishing nets and in those obtained from fish ponds is such that even sex determination to the unaided eye is difficult.

In order to have a statistically sound answer to the various problems, collection of a large amount of material involving a considerable lapse of time will be necessary. But meanwhile, some observations based on material obtained during the last few years are presented here which might partly fill the existing lacuna and will eventually lead to a fuller understanding of the various aspects in the biology of the fish.

Methods

The account is based mainly on *Chanos* collected off and on along the coast around Mandapam. The fish were dissected, the gonads taken out

and portions of them were fixed in Bouin's fluid for histological studies and the rest preserved in 5 per cent. formalin. Paraffin sections were stained in iron hæmatoxylin and counterstained with eosin. Mallory's collagen stain was also used. The determination of the number of ova in the gravid females was done in the fresh condition using the method of counting the eggs in a portion of known weight and then calculating the total number from the total weight of the ovaries. The ova diameter measurements were made in sections, more or less according to the procedure employed by De Jong (1939) and later workers on similar studies. In drawing the size frequency curves the diameter of about five hundred ova from each pair of ovaries were measured throughout at a magnification of 10×10 and grouped in 2 ocular micrometer division intervals when each division represented 14 micra. Histological descriptions of the ovary and the intraovarian eggs at various stages are broadly on the lines given by Franz (1908) and that of the testes after Craig-Bennet (1931). However, since cytological details are not of prime importance in this account several details have been omitted.

While referring to the length of the fish, the total length from the tip of snout to end of the longest lobe of the caudal fin is always given.

The Gonads

The external characters of the fish do not at any time permit us to distinguish the sex. In fish usually under about 500 mm. in total length the gonads themselves are hardly recognisable except as thin streaks of greyish-white, cord-like tissue loosely attached to the peritoneal wall dorsal to the alimentary canal and ventrolateral to the air-bladder. They extend almost to the whole length of the cœlomic cavity and may often be mistaken for a blood vessel. The differentiation into the ovary or the testis at this stage is difficult even under a microscope. Sections from the various regions also reveal only some undifferentiated mass of cells covered over by a coat of connective tissue and two blood vessels associated with it.

The Testis.—The descriptions are confined to two important stages, (i) the very early stage when the gonad becomes just recognisable histologically into the testis so that sex determination is possible in early juveniles and (ii) the very advanced or mature condition where the sperms are fully formed just prior to spawning. Descriptions of either of these are lacking in literature. The author has not come across intermediate stages in the maturation of the testis or the spent testis and thus it has not been possible to assign any different developmental stages of the testis as has been followed in the case of the ovary.

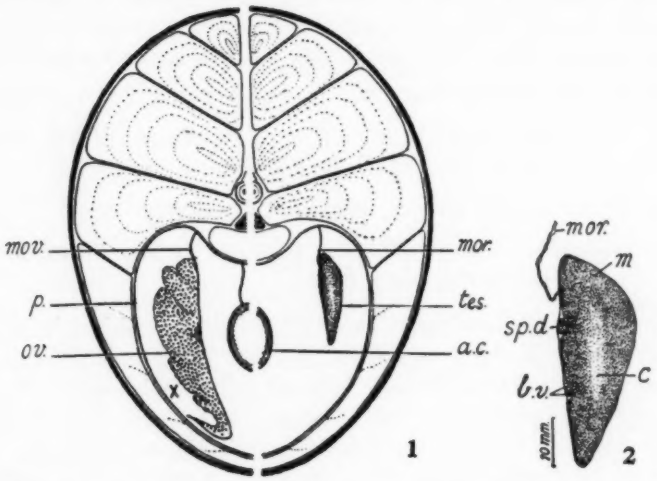
The differentiation of the undeveloped gonad into the testis is possible by studying sections and even this is apparent only in juveniles longer than about 500 mm. The testis remains as a thin cord of tissue having a maximum width of 2·5 mm. with a slightly pinkish-grey coloration even in fish up to about 900 mm. length. The thin mesorchium with which the testis is attached along its length to the peritoneal lining has scattered melanophores. Transverse sections of the testis at various levels reveal that the testicular material at this stage is confined to the anterior half of the elongated organ and posteriorly it is composed only of connective tissue and blood vessels.

Figure 1, Plate XVII shows a part of the testis of a fish of 690 mm. in a transverse section. The testis is provided with a fibrous connective tissue capsule covered by a mesothelial lining forming the tunica (*tu*) which is thicker toward the inner lateral side where a main artery and a vein are present. The attachment of the mesorchium is towards the inner dorsal margin. The mass of apparently undifferentiated cells of the testis is seen to be clumped together into small groups or lobules (*l.*) and are clearer along the peripheral regions (*cf.* Matthews, 1938). The cells are the resting sperm cells or the spermatogonia (*spg.*). The majority of the cells are large and possess clearly stained, round nuclei with an average diameter of 4 micra and small masses of darkly staining chromatin and one or two plasmosomes (Fig. 2, Pl. XVII). The cytoplasm is lightly stained and the cell-walls are somewhat clearly defined. In the testis of a fish of 830 mm. a few polygonal cells with small intensely staining round nuclei of only 2 micra in diameter and definitely granular cytoplasm may be seen. No mitotic figures or dividing spermatogonia have been found in any section of the testes taken from fish of varying lengths up to 830 mm. This might indicate that active spermatogenesis takes place only at later stages which are not represented in the present set of material. Further cytological details or the significance of the various types of cells and other descriptions in the progressive spermatogenesis are definitely outside the scope of this account.

The only other stage in the development of the testis obtained is the fairly mature testis which bears very little external resemblance to the stage described above. In this condition each testis appears as a rosy-white, elongated structure, broadest at about the middle and tapering at either ends (Fig. 3, Pl. XVII). The size of the testis varies in individual fish. In two specimens of 1,080 and 1,140 mm. length the testes measured 325×55 mm. and 280×43 mm. respectively excluding the vas deferens which itself is nearly half the length of the testis. The vas deferens is a continuation of the large spermatic duct and the vas deferens from each testis unite in the middle to

open to the outside by a common opening situated between the anus and the urinary aperture.

In this nearly mature condition the testis is heavy, somewhat soft to the touch and does not yield any milt under moderate pressure. Both the testes in a fish are equally developed. The general disposition of the testes (*tes.*) in the cœlomic cavity is schematically represented in the right half of Text-Fig. 1. The mesorchium (*mor.*) which is a thin, tough, translucent membrane devoid of much pigmentation is attached to the testis along its inner lateral side a little below the dorsal margin. On the same side are seen the spermatic



TEXT-FIGS. 1–2. Fig. 1. Schematic drawing of a transverse section of *Chanos* showing the gonads *in situ*. The right half represents that of the male and the left that of the female.

Fig. 2. Transverse section of a mature testis.

duct (*sp.d.*) a large artery and a vein lying superficially along the length of the organ (Text-Fig. 2). Transverse sections of mature testis when stained with iron hæmatoxylin will reveal an outer intensely stained cortical region (*c.*) and a relatively narrow, lightly staining central medullary zone (*m.*). The covering of the testis is composed of a thin connective tissue membrane while the testis itself is made up of a mass of spermatic tubules which open into the common collecting duct. These spermatic tubules are outlined by connective tissue fibres and blood capillaries. Fully formed spermatozoa (Fig. 4, Pl. XVII, *sp.*) present inside the tubules are densely accumulated along the peripheral regions of the testis which, when stained, give the apparent differentiation into the cortical and medullary regions. Each spermatozoa

has a tiny head, shaped almost like an inverted cup, and a long tail. In this advanced testis, besides the sperms various stages in the maturation of the spermatocytes (*spc.*) are also seen. The question of the presence of interstitial cells in the testis has not been gone into although it might prove significant in a detailed study of the cyclic changes in the testis.

The ovary.—The ovaries in the fish since they become definitely recognisable may be arbitrarily classified into four broad groups depending on the degree of development. However, it is to be made clear that this grouping does not in anyway correspond to the international standards in the classification of the maturing ovary, but has been adopted here in this preliminary account only for the sake of convenience in description and will necessarily have to be revised later. Thus the present material may be classified as:—

Stage I.—The earliest ovary which can be recognised histologically.

Stage II.—Where there is a uniform development of ova within the ovary and including all immature stages.

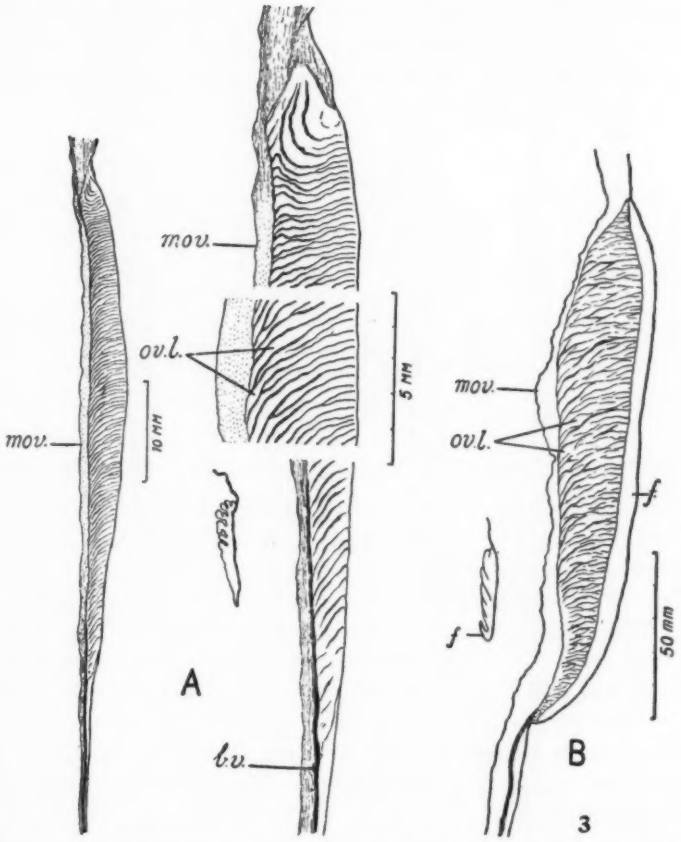
Stage III.—Enlarged ovaries with ripe intraovarian eggs.

Stage IV.—Spent ovaries after the eggs are shed and sometimes with re-forming early ova.

Stage I.—The obvious sign of ovarian activity by which the female reproductive organs can be distinguished, *viz.*, the formation of the oocytes is seen in fish which are about 500 mm. in length. In the thin gonad the oocytes are first produced nearer the anterior region as in the case of the testis, while posteriorly only connective tissue may be seen in the earlier stages. Fig. 5, Pl. XVIII shows a section taken from the anterior region of an early ovary where a few developing ova (*ocy.*) can be clearly recognised by their large size, almost ovoidal shape and finely granular cytoplasm which takes a deep stain with iron hæmatoxylin or acid fuchsin. The nucleus is also large and round with a prominent nucleolus. Comparatively smaller cells measuring on an average 7 micra are the oogonial cells (*og.*). The cytoplasm of these cells is clearer and the nuclei of these are also round and have scattered chromatin.

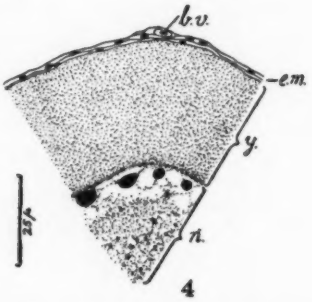
Stage II.—With the active development of the ova external morphological changes of the ovary gradually become apparent. There is slight enlargement of the ovary towards the anterior two-thirds where a pale pinkish colouration develops while the posterior region still remains attenuated. The ovarian tissue at this time is almost confined to the anterior major portion of the organ which becomes distinct. The mesovarium with which the ovary is attached to the dorsal peritoneum and also the formation of thin ovigerous amellæ (*ov.l.*) are visible at this stage. The general shape of the organ may

vary according to the extent of development of the ovigerous lamellæ, but in this Stage II is included all immature stages in the development of the ovary. Thus the shape may vary from a thin, flat, lanceolate structure as in Text-Fig. 3 A to a broader and more succulent organ as shown in Text-Fig. 3 B. The colour also varies correspondingly from a light pink to bright pink or pale orange-yellow depending on the maturation of the ova within them. In a fish of 990 mm. length the ovaries measured 160×27 mm. The ventral margin of the ovaries now develop a fold (*f.*) as is shown in the transverse section in Text-Fig. 3 B.



TEXT-FIG. 3. Immature ovaries of stage II. A. Entire ovary of a 502 mm. fish; enlarged drawing of the anterior, middle and posterior regions and also a transverse section showing the formation of the early ovigerous lamellæ. B. Entire ovary of a fish of 990 mm., a transverse section is also given alongside which shows the ventral marginal fold of the ovary.

Figures 5 and 6, Pl. XVIII show sections of ovaries of two fish 670 mm. and 990 mm. respectively. The difference in the state of maturity may be clear from these figures; Fig. 5 shows a very early condition while Fig. 6 exhibits a more advanced stage. The distinctive feature of the more developed ova, besides their larger size, are a granular cytoplasm which stains less intensely with acidophilic dyes, a large round nucleus of about 70 micra diameter within which are present a number of deeply staining nucleoli mostly arranged along the margin and a fine chromatin network in the middle (Text-Fig. 4). The outer membrane also becomes distinct. The ova of the less advanced stages are considerably smaller and have the same cytological nature as those of Stage I. In the 990 mm. fish the majority of the eggs have a diameter of 28–56 micra which represent the immature stock. Larger eggs varying in size up to about 140 micra are seen in this ovary and these larger ones are the maturing eggs.



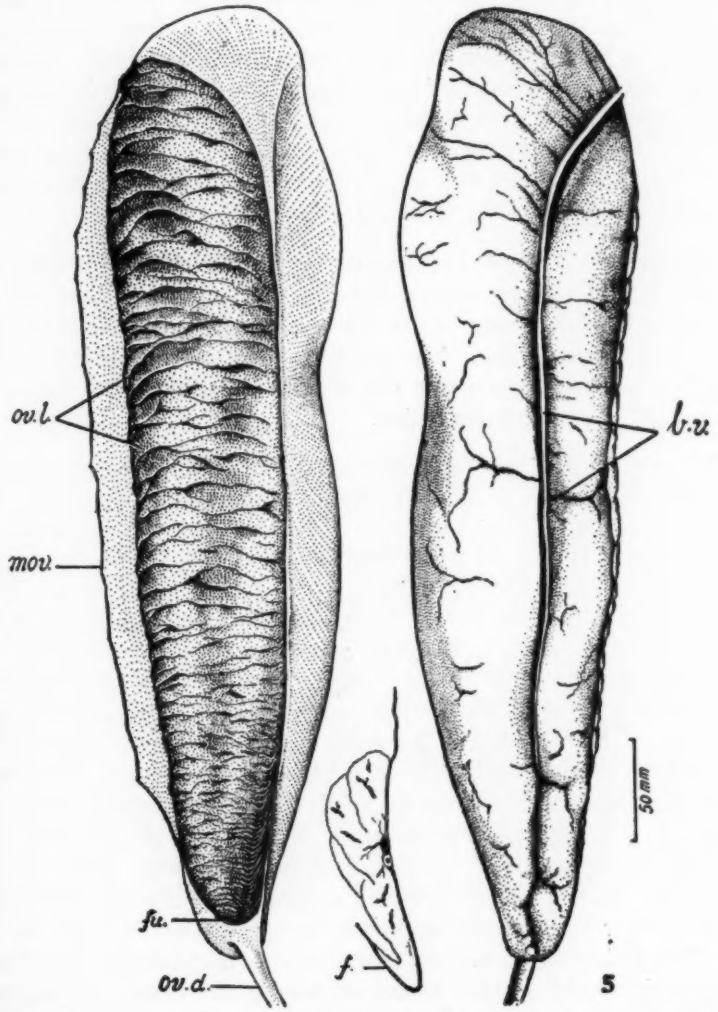
4

TEXT-FIG. 4. Section in part of an immature egg from the 990 mm. fish.

Stage III.—This stage is characterised by the nearly mature intraovarian eggs and represents the most advanced condition of ovarian development in the present collection. There, no doubt, exists a wide gap between this and the last stage described under II, but no intermediate stages have been obtained. The mature ovaries are considerably enlarged. The belly of a fish with such ovaries is highly distended and the pressure exerted by these on the abdominal wall is so enormous that as soon as a ventral incision of the body is made the ovaries force themselves out through the opening. They are bright yellow on account of the yolk-laden eggs which are round, somewhat translucent and visible through the thin transparent wall of the ovigerous lamellæ.

Text-Fig. 1 is here recalled, the left half of which shows in a diagrammatic way the disposition of the ovary within the cœlomic cavity as seen in a transverse section of the body of the fish. In the normal swimming position

of the fish the ovaries are suspended from the dorsal wall of the cœlome by the mesovarium which is continued as the ovarian wall and to which the ovigerous lamellæ are attached. Text-Fig. 5 shows the two sides of an ovary, the outer lamellar and the inner lateral side respectively. The ovigerous lamellæ are exposed and face the cœlomic wall, closely arranged in transverse



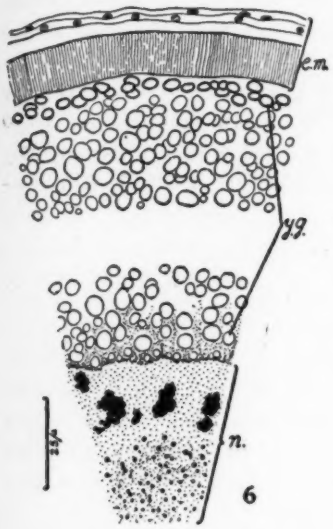
TEXT-FIG. 5. The two sides of a nearly mature ovary from a fish of 1,250 mm. and also a transverse section showing arrangement of the ovigerous lamellæ.

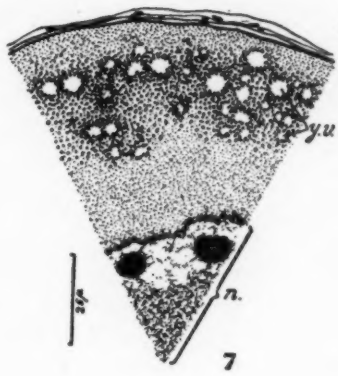
rows. Thus the ovary is of the gymnovarian type unlike in many other teleosts where the lamellæ are completely enclosed in a hollow sac and the type of ovary is called cystovarian. Each ovary in transverse section is seen to be slightly folded along the ventral side thereby cutting off a longitudinal space of the cœlomic cavity (marked X in Text-Fig. 1) into which the ovigerous lamellæ project. Towards the tapering posterior end of each ovary the ovarian wall together with the mesovarium forms a funnel-shaped structure serving as an 'oviducal funnel' (*fu.*) which leads to a short oviduct. The oviduct is about one-and-a-half inches long and each oviduct from either side unites in the middle and opens to the outside by a single genital aperture in front of the anus. The eggs are liberated into the cœlomic space adjacent to the ovary and are collected near the oviducal funnel from where they are conducted along the oviducts to the outside.

The two ovaries in a fish are more or less symmetrically developed (Fig. 8, Plate XVIII) although the shape and the size of the two may show some variation among individual fish. The Table on page 265 gives the measurements of the ovaries in three fish. The development of the ova within each ovary is uniform throughout and for ova diameter measurements samples were taken only from the middle region.

The maximum size of the mature eggs in these three pairs of ovaries varies from 728 to 756 micra (corresponding to 52 and 54 ocular micrometer units) in preserved material whereas when separated and measured in the fresh condition in normal saline their diameter varied from 800 to nearly 880 micra. Some amount of shrinkage during preservation as well as slight enlargement in saline solution may occur and thus the average size of the intraovarian eggs may be said to be of the order of 800 micra (0·8 mm.). Delsman (1929) gives 0·7 mm. as the average diameter of a mature intraovarian egg. The eggs when fresh are nearly translucent and become opaque on preservation. No oil globule is present but have finely divided yolk. In sections (Figs. 9 and 10, Pl. XIX; Text-Fig. 6) the yolk region which extends to about 280 micra all round the nucleus is seen to be composed of tiny spherules (*y.g.*) of 2–3 micra. The outermost layer of yolk granules take a comparatively deeper stain with acidophilic dyes and is often seen prominently just beneath the egg membranes. As maturation of the egg advances, this peripheral layer becomes less acidophilic and stains only like the rest of the yolk. The various layers like the theca externa, theca interna, granulosa and zona pellucida which together constitute the egg membrane covering the egg (*e.m.*) measure on an average 7 micra in thickness. The nucleus (*n.*) is about 140 micra and does not show a definite membrane. A number of nucleoli

arranged nearer the margin and scattered chromatin granules in the centre are also visible.





TEXT-FIGS. 6 & 7. FIG. 6. Part of a section of a nearly mature egg. Only about one-third of the yolk region is represented in the drawing. FIG. 7. Similar section of a reforming egg from a spent ovary of fish of 1,240 mm. long.

Stage IV.—The considerably reduced size of the ovaries with the lamellæ shrunken and falling apart, a pale reddish-grey colouration and flaccid nature, all very much unlike the mature ovaries, are almost ready indications of their spent condition. Fig. 11, Pl. XIX shows the section of a spent ovary. The majority of the ova shows a size range of 56–84 micra representing the primitive stock of eggs. A very small number of larger opaque eggs, which are not shown in the photograph, are also present which are those left over after the spawning and are in a state of reabsorption. In the case of fish of 1,260 mm. with similar spent ovaries, a number of eggs with an average diameter of 200 micra are noticed. Besides their larger size from the immature eggs these eggs show the formation of yolk vesicles (*y.v.*) and small yolk granules towards the peripheral zone indicating thereby a state of reformation (Text-Fig. 7).

The histological nature of the spent ovary, particularly of the 1,260 mm. fish, deserves special mention. Numerous small, orange-yellow round bodies are noticed on the surface of the ovaries. In sections these are found scattered throughout among the immature eggs and appear like disintegrating follicles (Fig. 12, Pl. XIX, *c.l.*). A closer study reveals that these are composed

of cells showing large vacuoles and round nuclei with scanty chromatin material. Similar structures, but in much fewer numbers, are also noticed in sections of the spent ovary of a 1,240 mm. fish but not seen in another spent fish of 1,203 mm. The histological nature of these peculiar bodies suggests a close resemblance to some of the stages in the formation of the "corpus luteum" described in *Rhodeus* by Bretschnieder and Duvyené de Wit (1947) and in *Fundulus* by Matthews (1938) and may be comparable to similar structures now described in other teleosts and a more detailed study of their histophysiology seems to be necessary in view of their probable hormonal function as reviewed by Hoar (1951).

EGG PRODUCTION

The approximate number of mature eggs in the ovaries of the gravid females is roughly from 2 million to about 5 million as may be seen from the table given below. Sunier (1922) estimated the number of eggs in a fish to be 57,00,000 which slightly exceeds the maximum obtained here, while Adams *et al.* (1932) found over 3 million eggs in a fish. No other figures seem to be available, but these in general seem to be in agreement with what has been observed in most other teleosts, *viz.*, that the total number of eggs produced by each fish is nearly proportional to its length.

SIZE AT MATURITY

Although the material on hand is insufficient for any conclusion, indications are that in the females the ovary starts active development with the formation of the early oocytes when the fish is about 500 mm. in length whereas in the males the spermatocytes begin to appear in the testis only after the fish reaches a size of more than 900 mm. Similarly measurements of males and females of about the corresponding stage of maturity show that the former are smaller than the latter thereby indicating that maturation of the testis proceeds at a faster rate than the ovary.

Since histological study of the ovaries of the three gravid females does not reveal the possibility of the fish having spawned previously it is assumed that these fish have been spawning for the first time. Thus it may be said that the first spawning takes place when the fish is about 1,100 mm. long (*ca.* 11·0 kg. in weight).

The probable age at which sexual maturity sets in is not known and an attempt was made by the author to estimate the approximate age of the mature fish. A study of the scales from different regions of the body, otoliths and other ossified centres was made and some very tentative conclusions have been reached. According to these preliminary observations, the author has reasons to suspect that the age of the female fish at first maturity is roughly

Table giving ovary dimensions and number of eggs

No.	Date	Length of fish (mm.)	Weight of fish (Kg.)	Ovary				
				Length (mm.)*	Width (mm.)*	Thickness (mm.)*	Weight (gm.)	Number of eggs in the two ovaries
1	5- 3-1954	1,105	11·79	342 355	110 98	28 23	824	21,18,000
2	7- 3-1954	1,210	11·80	408 425	110 85	25 25	1,010	34,33,000
3	7- 3-1954	1,250	13·60	435 438	108 120	27 30	1,405	48,96,000
4	16-10-1951	1,240	12·24	255 225	43 36	10 10	..	Spent
5	8- 2-1954	1,203	15·51	275 280	52 57	11 14	..	
6	24- 2-1954	1,260	14·60	302 293	53 68	15 15	..	
7†	..	1,120	11·90	..	..	..	1,304	57,00,000
8‡	..	..	..	..	..	..	..	30,00,000

* After preservation in formalin. Of the two sets of figures given the first one refers to the ovary of the right side of the fish and the other left.

† Data from Sunier (1922).

‡ Recorded by Adams *et al.* (1932).

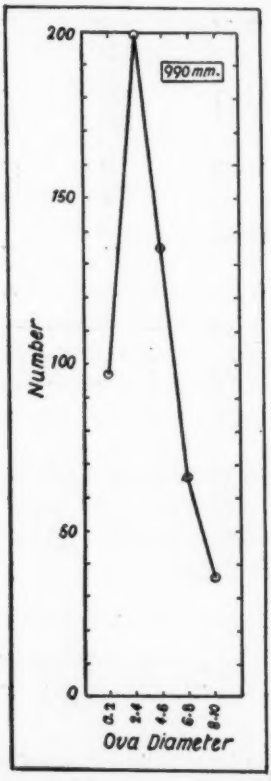
between 4 and 5 years (at about a length of 1,100 mm.). Similarly the females attain the early Stage II mentioned in this account when it is about 2 years old (*ca.* 600 mm. long) and takes another year to reach the late Stage II when the fish is about 900 mm. long.[1]

Type of Spawning and Spawning Period

A study of the ova diameter measurements of even these limited number of ovaries at different stages of development is of some help in interpreting

[1] Further collection of data relating to the question of age determination in the fish is in progress and it is hoped to publish a fuller account in due course.

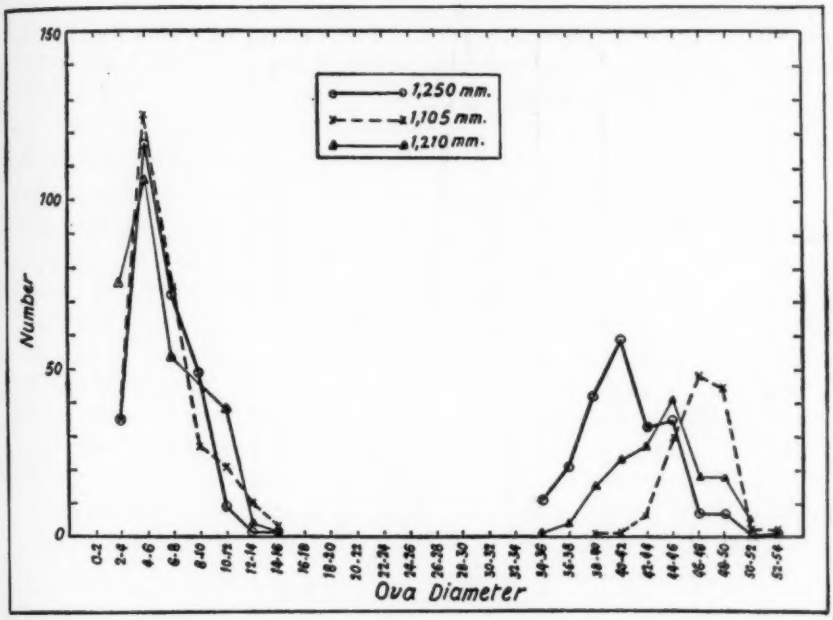
the frequency of spawning of the fish. In the ova size frequency curve (Text-Fig. 8) of a fish of 990 mm. length with the ovaries in a very early stage of maturation, only a single group comprising the immature eggs ranging in diameter up to about 140 micra is seen. Text-Fig. 9 gives the ova size frequencies in the ovaries of the three gravid females. These curves in general resemble that for the clupeoid fish *Chirocentrus dorab* whose spawning period has been discussed by Prabhu (1956). In all the curves there is a preponderance of primitive eggs whose diameter varies up to about 224 micra and with a single peak at a frequency of 56–84 micra (corresponding to 4–6 oc. mic. units in the graph). After this the curves show a gap until another distinct group of larger eggs occurs whose diameter varies between 504 and 756 micra (corresponding to 36 and 54 mic. units). The slight differences in the peak level in individual curves only indicate the state of maturity of these eggs in the different fish depending on the actual time at which these are to



8

TEXT-FIG. 8. Ova diameter frequencies in the ovary of *Chanos* of 990 mm. length.

be shed in each case. Thus this group represents those getting mature to be liberated during the spawning season. The absence of multiple modal curves and the presence of only two widely separated groups of eggs, *viz.*, the immature and mature ones, indicate that the fish has only one spawning season in a year. At the same time the indistinct minor modes within the group

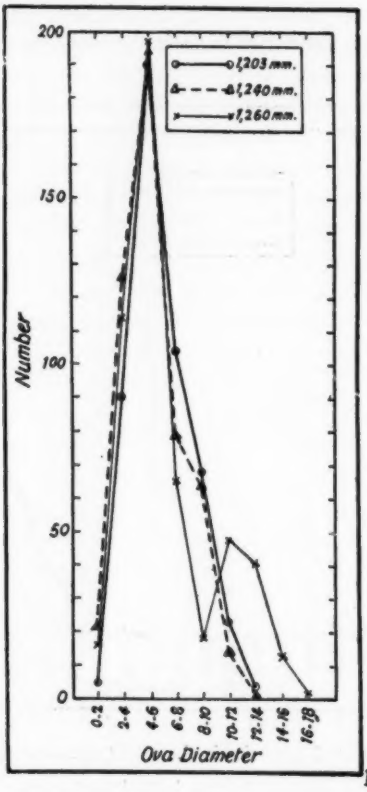


9

TEXT-FIG. 9. Ova diameter frequencies in the nearly mature ovaries of three fish.

of mature eggs as may be noticed in the individual curves and the wide range in size seem to indicate a tendency for some sort of fractional spawning within the season. But more material have to be examined and data on the growth rate of the maturing eggs studied over a period of 12 months is necessary for any conclusive evidence. The ova diameter measurements from the spent ovaries of three fish (Text-Fig. 10) also show more or less a single dominant group of immature ova except in the case of the 1,260 mm. fish. But whether the tendency to show up a minor peak corresponding to 10–12 mic. units in this instance is significant or not cannot be explained. Bunag (1951) who studied the spawning period of the fish from ova diameter measurements states that the spawning of *Chanos* along the Philippine coast is distinctly periodic. However, he seems to believe that the whole batch

of mature eggs is spawned at one time. In the unfortunate absence of a complete published account of his very interesting studies, further comparison is not possible.



10

TEXT-FIG. 10. Ova diameter frequencies in the spent ovaries of three fish.

If the mature ovaries do not suggest the possibility of two spawning seasons in a year for the fish we are confronted with a similar problem as experienced by Bunag (*op. cit.*). Thus a satisfactory explanation for the occurrence of the fry, though in small numbers, as reported by Panikkar *et al.* (1952) in the month of November and also by Ganapathy *et al.* (1950) that the fingerlings are collected near Madras and Pulicat during October to December, becomes difficult. Similarly Delsman (1929) and Schuster (1952) also mention two seasons during which the fry appear along the Indonesian coast where the October-November period is actually the major season for

fry collections and stocking. Since the two spawning seasons as is suggested by the actual fry collections do not fall at equal intervals in the course of twelve months, the question of the same fish breeding twice a year at unequal intervals may have to be ruled out as this might imply the presence of two types of eggs possessing two different maturation periods (one to be spawned in October–November and another to become mature again with a short period of 4 months by February–March), within the ovaries of the same fish, giving rise to an unequal cycle. This is somewhat unusual. On the other hand, the species as a whole might show two spawning seasons. This will then suggest the existence of another population responsible for giving rise to the subsidiary fry season in which case the necessity for a careful racial study of the species becomes obvious.

Observations through these years have shown that the earliest fry (of about 13 mm. long) appear along the Pamban coast for the first time usually between the second and third week of March although their peak season is from April–May. These fry along with fingerlings continue to occur in the succeeding months and have been collected in fair numbers up to the end of May and in smaller numbers even early in June. According to Delsman as quoted by Schuster (1952) the fry caught along the coast is roughly 2–3 weeks old. Thus we may reasonably assume that the spawning of the fish starts sometime by February and lasts until about the middle of May. The capture of both mature and spent fish within this period supports this. Similarly the wide range in the size of the group of maturing eggs also suggests a somewhat extended period of spawning.

Judging from the reports of Ganapathy *et al.* (1950) on the peak season of actual fry collection along the South Indian coast, one is inclined to suspect a slight shifting of the intensity of the season from April in the Pamban area to about June in the northern districts of the Coromandel coast. A similar change may be noticed towards the northern regions along the west coast also. If this is significant, it may be interesting to pursue the movements of the fry side by side with the change in factors such as water currents and hydrological conditions along these coastal regions.

Breeding Habits

It is through the classical work of Delsman (1929) that we now have, what seems to be, the only dependable description of the egg and early larvæ of the fish. His material was obtained from plankton in horizontal surface hauls in the Java Sea 15–17 miles from the coast where the sea is 11–22 fathoms (20–40 metres) deep. The only other collection is of Chacko (1950) who

also obtained the eggs from surface plankton collected around Krusadai Island in the Gulf of Mannar in South India. Since then the present author made a series of regular plankton hauls in these areas both at the surface and also at depths varying up to 6 feet. The collections were made during 1950–55 on months which cover the probable spawning period. Several eggs which conform to the known description of *Chanos* eggs were obtained in surface hauls while they were absent in the sub-surface collections. These were hatched in the laboratory and reared up to three days. A comparison of these with the corresponding stages given by Delsman showed differences in the manner of arrangement of the muscle fibres, pigmentation, etc.[2] Frey (1947) states that in *Chanos* "The females are said to average three to five million eggs, which are thought to be of the demersal type." The reasons for such a statement or the authority on which such a remark is based are not known and would have been interesting especially in view of the fact pointed out by Delsman (1929) that the number of eggs produced by the fish is so great that " .... we may safely draw the conclusion from it that the bandeng is a fish with pelagic eggs, as in fishes with demersal eggs or with some form of parental care not nearly such numbers are found."

Regarding the breeding behaviour of *Chanos*, the observations of Chacko (1951) which are of great interest have revealed that the fish spawns in selected places in the inshore regions of the coastal waters where there is slight reduction in salinity, which facilitate the drifting of the eggs and larval stages into the backwaters and tidal pools. The spawners which are 3–5 feet in length congregate in small shoals of 50- 60, during the nights of the new moon periods in the months of March, April and May. They are said to enter the backwaters if conditions are favourable. The present author, however, has failed to notice these phenomena in areas around Mandapam and the only two occasions when the mature ones were actually caught in the large shore seines were within about 3 miles from the coast.

In this connection, attention may also be drawn to a recent report by H. van Pel[3] that *Chanos* has been found to breed in a salt-water lake in New Caledonia of the Tonga Archipelago. Although this is against the existing belief that the fish do not breed in enclosed waters such a possibility cannot be entirely ruled out. In fish farms we seldom come across a fish with actively

---

[2] The help rendered by my colleagues Shri S. V. Bapat and Dr. P. Vijayaraghavan, in making plankton collections and a critical study of the newly hatched larvæ, is gratefully acknowledged. Both of them hold the view that these eggs and larvæ belonged to some other clupeoid fish.

[3] This report, made in 1956 to the Indo-Pacific Fisheries Council, was made available to me through the kindness of Dr. N. K. Panikkar, to whom the author's thanks are due.

developing gonad mainly because they are caught, perhaps for reasons of economy, long before this stage sets in. In one specimen of 630 mm. in length captured from the fish farms at Narakkal (Cochin), the ovaries showed distinctly developed lamellæ and the intraovarian eggs corresponded to the early stage described under Stage II. This might indicate that active development of the gonads even in enclosed waters is possible provided the fish are allowed to grow to a sufficient size over a longer period although their actual spawning in such confined space may be questionable. At least in experimental farms it may be interesting to have them reared to a maturing size so that it may throw some light on the physiological needs of the fish associated with sexual maturity. van Pel's observation and also the recent observations by the author (Tampi, 1957) on the food of the fish might be of significance in this line of investigation.

Summary

1. Some essential morphological and histological features of the very early and nearly mature testes in *Chanos* are described.

2. Similar accounts of four important stages in the maturation of the ovary and the intraovarian eggs are given.

3. A study of the ova diameter measurements in the limited material indicates that the fish normally spawns only once a year. The type of spawning in the species, egg production, size at maturity and breeding habits are discussed in the light of these observations. The probable lines on which further data may be usefully gathered are also briefly indicated.

Literature Cited

Adams, W., Montalben, H. R. and Martin, C. "Cultivation of bangos (*Chanos*) in the Philippines," *Philippine J. Sci.*, 1932, **47** (1), 1–35.

Bretschneider, L. H. and Duyvené de Wit, J. J. "Sexual endocrinology of non-mammalian vertebrates," *Monograph on the Progress of Research in Holland*, Amsterdam, 1947, 1–146.

unag, D. M. .. "Spawning habits of *Chanos chanos* (Forskål) based on ova diameter measurements of the developing ovarian eggs," *Tech. Paper* 165, *Indo-Pacific Fish. Coun.*, Bangkok (Abstract), 1951.

Chacko, P. I. .. "Marine plankton from waters around Krusadai Island," *Proc. Indian Acad. Sci.*, 1950, **31** (3), 162–74.

——— .. "Survey of Pulicat Lake with special reference to availability of *Chanos chanos*," *Prog. Rep. Madras Rur. Pisc. Scheme*, 1950–51, 1–33.

Chaudhuri, B. L. .. "Fauna of Chilka Lake, Fisheries, Part I," *Mem. Indian Mus.*, 1916, **5**, 405–39.

Craig-Bennet, A. .. "The reproductive cycle of the three-spined stickleback, *Gasterosteus aculeatus* Linn.," *Phil. Trans. Roy. Soc.*, 1931, **219 B**, 197–280.

De Jong, J. K. .. "A preliminary investigation of the spawning habits of some fishes of the Java Sea," *Treubia*, 1939, **17**, 307–27.

Delsman, H. C. .. "Fish eggs and larvæ from the Java Sea, *Chanos chanos* (Forskål)," *Ibid.*, 1929, **11**, 281–86.

Franz, V. .. "Die Epiproduktion der Scholle (*Pleuronectes platessa*)," *Wiss. Meeresunt, N.F.*, 1908, **9** (2), 63–141.

Frey, D. G. .. "The pond fisheries of Philippines," *J. Mar. Res.*, 1947, **6** (3), 247–58.

Ganapathy, S. V., Chacko, P. I., Srinivasan, R. and Krishnamurthy, B. "On the acclimatisation, transport and culture of some salt-water fishes in inland waters of the Madras State," *Indian Geogr. J.*, 1950, **25** (2), 1–15.

Hoar, W. S. .. "Hormones in fishes," *Univ. Toronto Biol. Ser.*, 1951, **59**, 1–51.

Matthews, S. A. .. "Seasonal cycle in the gonads of *Fundulus*," *Biol. Bull.*, 1938, **75** (1), 66–74.

Panikkar, N. K., Tampi, P. R. S. and Viswanathan, R. "On the fry of the milkfish *Chanos chanos* (F.), " *Curr. Sci.*, 1952, **21**, 18–19.

Prabhu, M. S. .. "Maturation of intraovarian eggs and spawning periodicities of some fishes," *Indian J. Fish.*, 1956, **3** (1), 59–90.

Schuster, W. H. .. "Fish culture in the brackish-water ponds of Java," *Indo-Pacific Fish. Counc.*, Special Publication, 1952, **1**, 1–143.

Sunier, A. J. .. "Marine fish ponds of Batavia," *Treubia*, 1922, **2**, 159–400.

Tampi, P. R. S. .. "Some observations on the food of *Chanos chanos* (Forskål)," *Indian J. Fish.*, 1957 (In press).

EXPLANATION OF PLATES

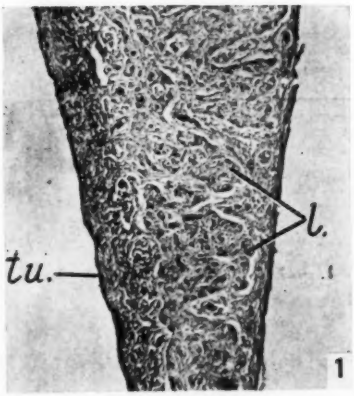
PLATE XVII

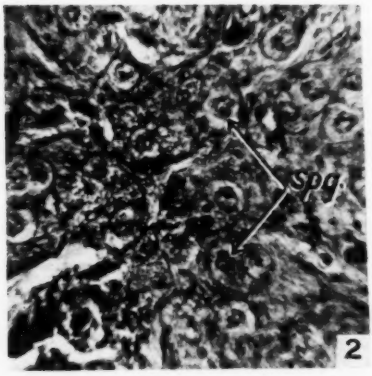
FIG. 1. Part of a transverse section of a very early testis from a fish of 690 mm. showing a few lobules of spermatogenic cells, ×55.

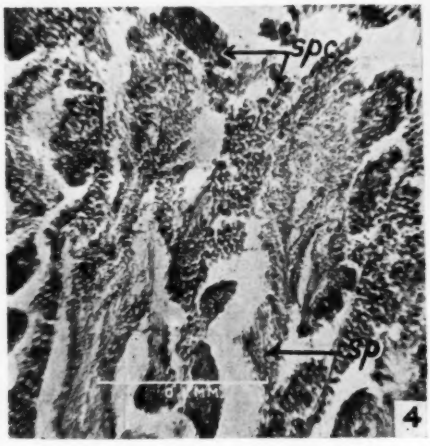
FIG. 2. Spermatogenic cells from a 830 mm. fish, ×550.

FIG. 3. Photograph of the mature testes of a 1,140 mm. fish, ×1/3.

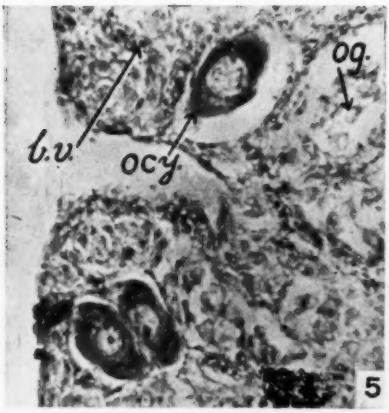
FIG. 4. Various stages of spermatocytes and fully formed spermatozoa in a spermatic tubule of a mature testis, ×260.

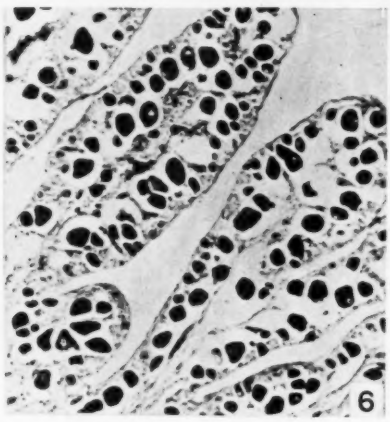

l.
tu.
1

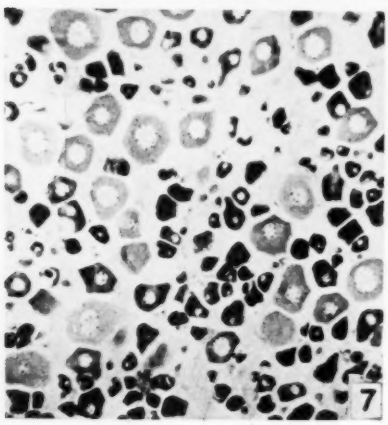

spg.
2


spc.
sp.
4

tes
v.d.
3

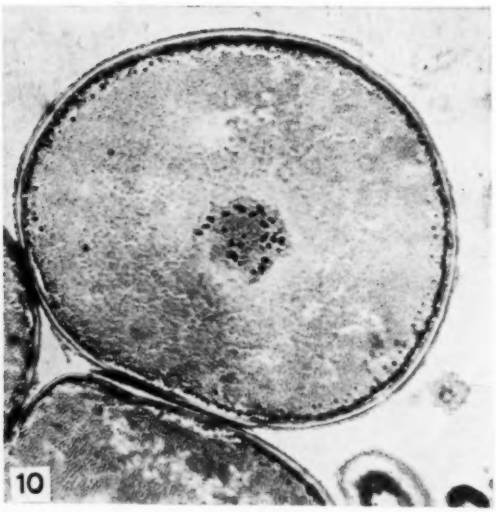

og.
b.v.
ocy.
5

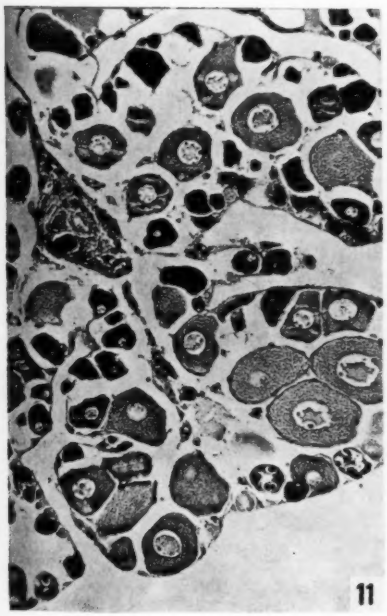

6


7

8

9


10


11

c.l.
12

PLATE XVIII

FIG. 5. Oogonial cells and oocytes from the earliest recognisable ovary of a fish of 500 mm. of Stage I, ×500.

FIG. 6. A few ovigerous lamellæ from a 670 mm. fish of early Stage II, ×86.

FIG. 7. Part of the ovary of a 990 mm. fish of Stage II, but more advanced than in Fig. 6, ×55.

FIG. 8. Photograph of the mature ovaries from a fish of 1,250 mm. *ca.* ×1/3.

PLATE XIX

FIG. 9. Section of a nearly mature ovary of Stage III from a 1,210 mm. fish showing a few intra-ovarian eggs, ×17.

FIG. 10. Section of a nearly mature intraovarian egg, ×100.

FIG. 11. Section of a spent ovary of a fish of 1,240 mm., ×90.

FIG. 12. Section of ovary of the fish of 1,260 mm. length. Note the peculiar bodies referred to in the text, ×90.

KEY TO LETTERING

b.v.	Blood vessel.	*ov.*	Ovary.
c.	Cortex.	*ov.d.*	Oviduct.
c.l.	"Corpus luteum".	*ov.l.*	Ovigerous lamellæ.
e.m.	Egg membrane.	*sp.*	Spermatozoa.
f.	Marginal fold of ovary.	*spc.*	Spermatocytes.
fu.	'Oviducal funnel'.	*spg.*	Spermatogonial cells.
l.	Lobules of spermatogenic cells.	*sp.d.*	Spermatic duct.
m.	Medulla.	*tes.*	Testis.
mor.	Mesorchium.	*tu.*	Tunica.
mov.	Mesovarium.	*v.d.*	Vas deferens.
n.	Nucleus.	*y.*	Yolk.
ocy.	Oocytes.	*y.g.*	Yolk granules.
og.	Oogonial cells.	*y.v.*	Yolk vesicles.

ASSIMILATION OF DISACCHARIDES BY SOME FUNGI CAUSING "LEAF SPOT" DISEASES

By R. N. Tandon and K. S. Bilgrami
(*Department of Botany, University of Allahabad*)

Received December 26, 1956
(Communicated by Dr. Shri Ranjan, F.A.SC.)

Introduction

Assimilation of disaccharides by the micro-organisms generally depends upon the production of necessary hydrolytic enzymes. Non-utilization of these complex sugars is usually associated with lack of such enzymes. Instances are known where the complex sugars may not be utilized by an organism even though its hydrolytic products may support good growth. Thus Margolin (1942) found that *Syncephalastrum racemosum* could utilize the hydrolytic products of lactose (*viz.*, glucose and galactose), but it was incapable of using lactose. According to him the non-utilization of lactose was due to the inability of that organism to synthesize lactase. Mandels (1954), however, suspected that in some cases the disaccharides could be assimilated even without being hydrolysed. He suggested that the spores of the fungus *Myrothecium verrucaria* did not use sucrose through a hydrolytic pathway. Smith (1949) also obtained almost similar results with *Marasmius chordalis* and according to him, this organism attacked cellobiose by a route that involved neither preliminary hydrolysis nor phosphorylation. The observations of Smith (1949) and Mandels (1954) indicate the direct utilization of disaccharides by some fungi, but such records of direct assimilation of complex sugars are rare. Hawker and Chaudhari (1946) reported that the rate of inversion of sucrose determined the amount of growth of *Podospora* sp., *Pyronema confluens* and *Chætomium cochliodes*. It is thus evident that not only the pathway of utilization of disaccharides may vary with different fungi, but even their rate of assimilation may depend on the rate of hydrolysis of the sugar.

An attempt has, therefore, been made to undertake chromatographic studies to establish the pathway of utilization (hydrolytic or non-hydrolytic) of some common disaccharides (*viz.*, sucrose, maltose and lactose) by three leaf spot causing fungi.

Materials and Methods

The three fungi, *viz.*, *Phyllosticta cycadina* (Pass.), *Phyllosticta artocarpina* (Syd. *et* Butl.) and *Pestalotia mangiferæ* (Butl.), which were responsible

for the leaf spot diseases of *Cycas revoluta*, *Artocarpus heterophyllus* and *Mangifera indica* respectively, were isolated from the infected leaves of the above mentioned hosts. The methods of isolation, purification and subculturing were similar to those of Tandon and Bilgrami (1954). Asthana and Hawker's medium A* which has glucose as the only source of carbon was selected as the basal medium. Glucose was replaced by different disaccharides and mixture of their hydrolytic products. Care was taken to include the same amount of carbon (*i.e.*, 2 gm. per 1,000 ml. in each case). Pyrex glass wares and purest available chemicals were used. Fixed quantity of medium (*viz.*, 25 ml.) was taken in each 150 ml. conical flasks which were fractionally sterilized by steaming for half an hour daily for three successive days. Solutions containing these sugars were daily inoculated for 15 days with different organisms by agar disc method of Garrett (1936). The fungal mat from each set was filtered separately on the sixteenth day when one to fifteen days old cultures were available. The calculated dry weight was used as the quantitative measure for daily growth. The filtrate from each set was chromatographically analysed to detect the presence of the sugars. The method described by Ranjan *et al.* (1955) was used for this purpose. A circular piece of Whatman filter-paper No. 1, diameter 27 cm. having 12 radial sectors separated by 12 radial cuts was used. Drops of known volume (0·005 ml.) were taken from the filtrate of each day and they were placed at the positions located for this purpose (just above the numbers 1, 2, 3, ... 10, etc.). Sometimes index solutions of known sugars were also kept on the same chromatogram because this could facilitate the identification and comparison of bands. After placing the drops, the chromatograms were run with *n*-butanol-acetic acid and water (4 : 1 : 5). For separation of glucose and galactose (which gave a common band with the above solvent) *n*-butanol-pyridine and water (60 : 40 : 30) were used as the developing solvent. The chromatograms had single paper wick (4×2 cm.) and were run for about 7 hours in the above solvent. They were then dried at room temperature for 2–3 hours and were subsequently sprayed with a mixture of aniline-diphenyl amine phosphate (5 volumes of 4% aniline, 5 volumes of 4% diphenyl amine and 1 volume of phosphoric acid). After spraying, the chromatograms were left at room temperature for 3–4 hours and were then placed in an electric oven at 110° C. for 90 seconds. The average R_f values of various sugars were determined and they have been recorded in brackets at appropriate places in the text.

---

* KNO_3 3·5 gm., dextrose 5·0 gm., KH_2PO_4 1·75 gm., $MgSO_4 \cdot 7H_2O$ 0·75 gm., distilled water 1 litre.

The daily dry weight of these organisms on different disaccharides has also been recorded and their growth has been compared with the growth obtained on a mixture of corresponding monosaccharides, which were formed by the hydrolysis of various disaccharides.

OBSERVATIONS

1. *Assimilation of Sucrose* (R_f 0·61).—Chromatographic analysis of all the three hosts showed the presence of this sugar as well as of its hydrophytic products (*viz.*, glucose and fructose). Most of the fungi hydrolyse this sugar before assimilation. Daily chromatographic analysis of the medium revealed that the present organisms also utilized sucrose through a hydrolytic pathway. The rate of conversion of sucrose, however, varied with the organisms. It was observed that between 2nd and 3rd day, the two species of *Phyllosticta* converted the entire sucrose into a mixture of glucose (R_f 0·66) and fructose (R_f 0·69) and within that period it (sucrose) totally lost its identity in the medium. The same chromatograms also established that both these fungi fully assimilated glucose, three days earlier than fructose. The hydrolysis rate of this sugar (*viz.*, sucrose) by *Pestalotia mangiferæ* was much slower because it could not be hydrolysed completely even upto 7th day. It was also noticed that both the hydrolytic products of sucrose were simultaneously consumed on the 15th day.

The daily dry weight growth rate of these fungi on sucrose as well as a mixture of its hydrolytic products is summarized in Table I.

It is evident from the table that growth rate of both the species of *Phyllosticta* increased upto 5th day but in each case there was a progressive fall from the 6th day. The dry weight of *P. cycadina* increased upto 15th day while that of *P. artocarpina* increased upto 16th day. *Pestalotia mangiferæ*, however, did not show any growth upto 3rd day. Chromatographic analysis of the medium had indicated that sucrose was not hydrolysed by *Pestalotia mangiferæ* upto that day (3rd day). Table I also shows that the growth rate of *Pestalotia mangiferæ* increased upto 6th day and then it decreased. The dry weight of the organism, however, continued to increase upto the 16th day. These organisms were also grown on a mixture of glucose and fructose and it was observed that all the three fungi exhibited almost similar behaviour on this mixture as on sucrose except for the fact that *Pestalotia mangiferæ* which did not show any growth on sucrose upto 3rd day was capable of producing mycelium from the 2nd day.

2. *Assimilation of Maltose* (R_f 0·55).—Maltose on hydrolysis yields glucose only. Chromatographic analysis of the medium containing this

TABLE I

Showing daily dry weight (in mgm.) and growth rate of Phyllosticta cycadina, P. artocarpina *and* Pestalotia mangiferæ *on sucrose as well as a mixture of its hydrolytic products*

	DAYS															
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Phyllosticta cycadina—																
1 Daily dry wt. on sucrose ..	0·0	3·3	·0	27·2	40·0	50·5	59·5	67·7	75·0	81·5	84·5	86·8	88·0	88·5	88·8	88·8
2 Daily dry wt. on mixture of glucose and fructose	0·0	3·9	15·3	29·0	44·0	54·0	63·3	71·5	78·0	84·0	83·3	88·0	89·0	89·7	90·0	90·0
3 Growth rate on sucrose ..	0·0	3·3	11·7	12·2	12·8	10·5	9·0	8·2	7·3	6·5	3·0	2·3	1·2	0·5	0·3	..
4 Growth rate on mixture of glucose and fructose	0·0	3·9	11·4	13·7	15·0	10·0	9·3	8·2	6·5	6·0	2·3	1·7	1·0	0·7	0·3	..
Phyllosticta artocarpina—																
1 Daily dry wt. on sucrose ..	0·0	4·3	14·0	26·0	42·3	51·8	60·8	69·0	77·2	85·0	90·5	92·5	94·3	95·5	96·5	97·0
2 Daily dry wt. on mixture of glucose and fructose	0·0	3·4	12·5	25·1	41·5	52·0	60·2	68·0	75·5	82·3	85·5	88·5	90·8	92·8	94·0	94·5
3 Growth rate on sucrose ..	0·0	4·3	9·7	12·0	16·3	9·5	9·0	8·2	8·2	7·8	5·5	2·0	1·8	1·2	1·0	0·5
4 Growth rate on mixture of glucose and fructose	0·0	3·4	9·1	12·6	16·4	10·5	8·2	7·8	7·5	6·8	3·2	3·0	2·3	2·0	1·2	0·5
Pestalotia mangiferæ—																
1 Daily dry wt. on sucrose ..	0·0	0·0	0·0	4·6	9·5	17·0	23·1	28·0	32·5	36·5	40·1	43·5	46·8	49·8	52·7	55·5
2 Daily dry wt. on mixture of glucose and fructose	0·0	2·5	10·5	19·4	30·5	37·5	44·0	48·2	50·5	52·5	54·2	55·5	56·5	57·3	57·5	57·6
3 Growth rate on sucrose ..	0·0	0·0	0·0	4·6	4·9	7·5	6·1	4·9	4·5	4·0	3·6	3·4	3·3	3·0	2·9	2·8
4 Growth rate on mixture of glucose and fructose	0·0	2·5	8·0	8·9	11·1	7·0	6·5	4·2	2·3	2·0	1·7	1·3	1·0	0·8	0·2	0·1

sugar revealed the formation of glucose during its assimilation. The rate of hydrolysis of maltose also varied with the organism. Culture medium inoculated with *P. cycadina* showed the presence of glucose (R_f 0·66) on the 3rd day but it disappeared on the 8th day. Traces of maltose were also observed upto 8th day. In the medium on which *P. artocarpina* was growing, the formation of glucose was observed on the second day. In this case presence of glucose was recorded upto 9th day, while that of maltose upto 7th day only. The rate of hydrolysis of maltose by *Pestalotia mangiferæ* was very slow. Glucose appeared on the 6th day, and persisted upto 11th day while maltose was present only upto 10th day. During the process of assimilation of maltose by these fungi, it was observed that besides glucose one more sugar was also formed in the medium. The R_f value of this band (band III) was 0·18 which indicated it to be maltotriose (an oligosaccharide) and it was suspected that this sugar was synthesized during the hydrolysis of maltose. For further confirmation of this band the chromatogram was sent to Professor K. V. Giri* at Bangalore. Giri (1955) in a personal communication also agreed that the particular band (band III) was of maltotriose.†

The daily dry weight of these organisms on maltose and its hydrolytic product (glucose) is recorded in Table II.

Table II indicates that growth rate of both the species of *Phyllosticta* increased upto 5th day, while that of *Pestalotia mangiferæ* continued to increase upto 7th day. The dry weight of *Phyllosticta cycadina* and *Pestalotia mangiferæ* increased upto 15th day. *Phyllosticta artocarpina*, however, showed an increase in the dry weight upto 16th day. No growth of *Pestalotia mangiferæ* was evident upto 3rd day. This appears to be connected with delayed and slow hydrolysis of maltose by this fungus. A comparison of growth of these organisms on maltose with that of glucose showed that all the three organisms attained better mycelial growth on maltose than on glucose. Chromatographic analysis of the medium containing glucose only revealed that it was consumed in 9, 12, and 14 days by *Phyllosticta cycadina*, *P. artocarpina* and *Pestalotia mangiferæ* respectively.

3. *Assimilation of Lactose* (R_f 0·51).—Lactose has not been reported to be present in plants. Hydrolysis of lactose with acids or lactase yields a molecule each of glucose and galactose. Daily chromatographic analysis of the medium showed that this sugar was not hydrolysed by any of the three

---

* Professor of Biochemistry, Indian Institute of Science, Bangalore-3.

† In spite of best efforts, this sugar could not be obtained in synthetic form and it was, therefore, not possible to include it for detailed studies or to compare the band obtained with the band of maltotriose.

TABLE II

Showing daily dry weight (in mgm.) and growth rate of Phyllosticta cycadina, P. artocarpina *and* Pestalotia mangiferæ *on maltose as well as its hydrolytic product (glucose)*

		Days														
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Phyllosticta cycadina—																	
1 Daily dry wt. on maltose	..	0·0	5·0	19·0	35·0	53·0	66·5	74·5	80·0	84·0	86·0	87·0	87·9	88·5	89·0	89·3	89·3
2 Daily dry wt. on glucose	..	0·0	3·3	13·5	26·0	39·2	53·0	63·5	69·5	74·3	78·5	82·7	83·3	83·8	84·1	84·2	84·2
3 Growth rate on maltose	..	0·0	5·0	14·0	16·0	18·0	13·5	8·0	5·5	4·0	2·0	1·0	0·9	0·6	0·5	0·3	..
4 Growth rate on glucose	..	0·0	3·3	10·2	12·5	13·2	13·8	10·5	6·0	4·8	4·2	4·2	0·6	0·5	0·3	0·1	..
Phyllosticta artocarpina—																	
1 Daily dry wt. on maltose	..	0·0	4·5	17·0	32·0	52·0	63·0	73·0	81·5	87·5	92·5	97·0	100·0	102·5	104·5	105·5	106·0
2 Daily dry wt. on glucose	..	0·0	3·5	12·0	22·5	34·6	47·0	59·5	69·0	76·2	82·0	87·0	91·3	91·7	92·0	92·2	92·2
3 Growth rate on maltose	..	0·0	4·5	12·5	15·0	20·0	11·0	10·0	8·5	6·0	5·0	4·5	3·0	2·5	2·0	1·0	0·5
4 Growth rate on glucose	..	0·0	3·5	8·5	10·5	12·1	12·4	12·5	9·5	7·2	5·8	5·0	4·3	0·4	0·3	0·2	..
Pestalotia mangiferæ—																	
1 Daily dry wt. on maltose	..	0·0	0·0	0·0	6·0	17·5	33·5	55·0	63·0	67·0	69·4	71·5	73·2	74·5	75·3	75·5	75·5
2 Daily dry wt. on glucose	..	0·0	2·4	8·6	15·1	22·2	20·0	38·5	42·5	46·5	50·0	53·3	56·0	58·2	60·2	62·2	63·5
3 Growth rate on maltose	..	0·0	0·0	0·0	6·0	11·5	16·0	21·5	8·0	4·0	2·4	2·1	1·7	1·3	0·8	0·2	..
4 Growth rate on glucose	..	0·0	2·4	6·2	6·5	7·1	7·8	8·5	4·0	4·0	3·5	3·3	2·7	2·2	2·0	1·8	1·5

TABLE III

Showing daily dry weight (in mgm.) and growth rate of Phyllosticta cycadina, P. artocarpina *and* Pestalotia mangiferæ *on lactose as well as a mixture of its hydrolytic products*

	DAYS															
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Phyllosticta cycadina—																
1 Daily dry wt. on lactose ..	0·0	2·3	7·5	13·0	20·0	25·0	29·3	32·0	34·0	35·6	36·5	37·2	37·7	38·0	38·2	28·2
2 Daily dry wt. on mixture of glucose and galactose	0·0	4·5	13·0	22·0	33·0	47·5	56·0	64·0	70·0	73·5	76·6	79·0	81·0	82·3	83·0	83·5
3 Growth rate on lactose ..	0·0	2·3	5·2	5·5	7·0	5·0	4·3	2·7	2·0	1·6	0·9	0·7	0·5	0·3	0·2	..
4 Growth rate on mixture of glucose and galactose	0·0	4·5	8·5	9·0	11·0	14·5	8·5	8·0	6·0	3·5	3·1	2·4	2·0	1·3	0·7	0·5
Phyllosticta artocarpina—																
1 Daily dry wt. on lactose ..	0·0	3·5	7·5	12·0	17·4	23·5	30·5	36·2	40·2	43·3	46·0	48·0	49·2	49·9	50·4	50·5
2 Daily dry wt. on mixture of glucose and galactose	0·0	4·0	14·0	26·0	40·0	52·0	61·0	68·5	76·0	83·1	89·5	92·2	94·5	96·5	98·0	98·5
3 Growth rate on lactose ..	0·0	3·5	4·0	4·5	5·4	6·1	7·0	5·7	4·0	3·1	2·7	2·0	1·2	0·7	0·5	0·1
4 Growth rate on mixture of glucose and galactose	0·0	4·0	10·0	12·0	14·0	12·0	9·0	7·5	7·5	7·1	6·4	2·7	2·3	2·0	1·5	0·5
Pestalotia mangiferæ—																
1 Daily dry wt. on lactose ..	0·0	2·9	6·8	11·0	14·5	18·0	21·2	24·0	26·0	27·6	28·7	29·6	30·4	30·9	31·2	31·2
2 Daily dry wt. on mixture of glucose and galactose	0·0	3·5	9·5	16·5	24·0	29·0	33·8	38·3	41·3	44·1	46·3	48·3	50·1	51·6	52·8	53·5
3 Growth rate on lactose ..	0·0	2·9	3·9	4·2	3·5	3·5	3·2	2·8	2·0	1·6	1·1	0·9	0·8	0·5	0·3	..
4 Growth rate on mixture of glucose and galactose	0·0	3·5	6·0	7·0	7·5	5·0	4·8	4·5	3·0	2·8	2·2	2·0	1·8	1·5	1·2	0·7

organisms and glucose or galactose were, therefore, not formed in the culture medium. The above results also established that lactose was finished on 9th and 12th day by *Phyllosticta cycadina* and *P. artocarpina* respectively, while *Pestalotia mangiferæ* could not utilize all the lactose of the medium even in 15 days. The daily dry weight and growth rate of these organisms on lactose and a mixture of its hydrolytic products is recorded in Table III.

It is evident from Table III that the growth rate of *P. cycadina* and *P. artocarpina* increased upto 5th and 7th day respectively, while the rate of growth of *Pestalotia mangiferæ* was never very high and was not significantly different between 2nd and the 8th day. These fungi showed much better growth on a mixture of glucose and galactose (hydrolytic products of lactose) than on lactose. Chromatographic analysis of the medium containing this mixture revealed that all the three fungi assimilated glucose (R_f 0·67) one day earlier than galactose (R_f 0·65). Glucose was assimilated in 9, 11 and 13 days by *P. cycadina*, *P. artocarpina* and *P. mangiferæ* respectively.

Discussion

Present investigations clearly showed that sucrose and maltose were good carbon sources for all the three organisms. Similar results with these two sugars were also obtained by Herrick (1940), Blank and Talley (1941), Cantino (1949), Fergus (1952), Wolf (1953) and Agarwal (1955) for the fungi investigated by them. Daily chromatographic analysis of the medium indicated that all the present fungi assimilated sucrose and maltose through a hydrolytic pathway. The results further showed that out of the two hydrolytic products of sucrose, glucose was assimilated three days earlier than fructose by the two species of *Phyllosticta*, though both glucose and fructose were present in their hosts. Separate investigations by Bilgrami (1956) also confirmed these results and it was observed that both the species of *Phyllosticta* gave better mycelial growth on glucose than on fructose.

Pestalotia mangiferæ differed from the two species of *Phyllosticta*, because it was a slow hydrolysing fungus, and it consumed both the hydrolytic products of sucrose simultaneously. Hawker (1947) reported that the amount of mycelium produced by *Melanospora destruens* was different when this fungus was grown on sucrose or on a mixture of glucose and fructose. Her results indicated that it was not always essential that a complex carbohydrate (*e.g.*, sucrose) or its hydrolytic products (*e.g.*, mixture of glucose and fructose) should always be equally good sources. The results obtained with the present organisms differed from those of Hawker (1947). It was observed that there was no significant difference in dry weight of these fungi on sucrose or on a mixture of its hydrolytic products. Records on daily rate of assimila-

tion of sucrose had indicated that *Pestalotia mangiferæ* showed slightly delayed response on sucrose than on a mixture of glucose and fructose. It appears that this may be due to delayed and slow activity of the enzyme invertase. Chromatographic analysis of the medium on which *P. mangiferæ* was growing also confirmed that glucose and fructose were not formed in the culture medium till 3rd day and growth appeared after that period. Thus it is also clear that sucrose is not assimilated by these organisms till it is hydrolysed. *Pestalotia mangiferæ* showed delayed response on maltose also, as the growth of this fungus started on the 4th day, though glucose (hydrolytic product of maltose) was detected in the medium on the 6th day. It appears that whatever glucose was formed on the 4th and 5th day was entirely taken up by this organism, so that no trace was left in the medium and hence it could not be detected chromatographically. The behaviour of lactose was different from that of sucrose or maltose as it was not only a poor source of carbon, but was assimilated through a non-hydrolytic pathway and in this respect the present results seem to confirm the observations of Margolin (1942).

The present results also established that the different disaccharides may differ in their behaviour, because it was clearly observed that the growth on sucrose was similar, on maltose it was better and on lactose it was poorer than on their hydrolytic products.

Chromatographic studies showed the presence of maltotriose (an oligosaccharide) which appears to have been synthesized during the process of assimilation and which seems to play an important role in making maltose a better carbon source than its hydrolytic product. It may be mentioned that recently various workers, including Giri, Nigam and Srinivasan (1954) as well as Buston and Jabbar (1954), have reported that various fungi could play an important part in enzymic synthesis of oligosaccharides from disaccharides. The exact role of maltotriose in assimilation of maltose is, however, not clearly understood by any investigator. The present investigations indicate that these fungi were capable of synthesizing oligosaccharide only from maltose and not from the other two disaccharides, *viz.*, sucrose and lactose.

SUMMARY

Phyllosticta cycadina, P. artocarpina and *Pestalotia mangiferæ* were isolated from the leaf spot diseases of *Cycas revoluta, Artocarpus heterophyllus* and *Mangifera indica* respectively. A detailed study of the assimilation of sucrose, maltose and lactose by these organisms was undertaken. Sucrose and maltose were assimilated through a hydrolytic pathway. *Pestalotia mangiferæ* utilized both glucose and fructose simultaneously, but the two

species of *Phyllosticta* behaved differently and consumed glucose three days before the other hydrolytic product of sucrose (*viz.*, fructose). Synthesis of an oligosaccharide (maltotriose) was recorded during the assimilation of maltose. Lactose was used through a non-hydrolytic pathway and was found to be a poor source but a mixture of its hydrolytic products supported good growth.

Acknowledgments

The authors are indebted to Professor Shri Ranjan, Head of the Botany Department, Allahabad University, for providing the necessary laboratory facilities. They also wish to express their thanks to Prof. Giri for sending his opinion on the chromatograms showing the presence of maltotriose.

References

1. Agarwal, G. P. .. "Cultural and pathological studies of some *Fungi Imperfecti*," *D. Phil. Thesis, University of Allahabad*, 1955.
2. Bilgrami, K. S. .. "Pathological and physiological studies of some fungi-causing leaf spot diseases," *Ibid.*, 1956.
3. Blank, L. M. and Talley, P. J. "The carbon utilization and carbohydrate activity of *Phymatotrichum omnivorum*," *Amer. J. Bot.*, 1941, **28**, 564–69.
4. Buston, H. W. and Jabbar, A. "Synthesis of oligosaccharides by *Chaetomium globosum*," *Chem. and Indust. (London)*, 1954, **2**, 48.
5. Cantino, E. C. .. "The physiology of aquatic Phycomycete *Blastocladia pringsheimii*," *Amer. J. Bot.*, 1949, **36**, 95–112.
6. Fergus, C. L. .. "The nutrition of *Penicillium digitatum*," *Mycologia*, 1952, **44**, 183–92.
7. Garrett, S. D. .. "Soil condition and take all disease of wheat," *Ann. appl. Biol.*, 1936, **93**, 667–99.
8. Giri, K. V. .. Personal Communication, 1955.
9. ———, Nigam, V. N. and Srinivasan, K. S. "Enzymic synthesis of oligosaccharides from sucrose and lactose by *Aspergillus flavus*," *J. Indian Inst. Sci.*, 1954, **36**, 259–66.
10. Hawker, L. E. .. "Effect of temperature of storage on the rate of loss of fertility of stock cultures of *Melanospora destruens*," *Nature (London)*, 1947, **159**, 136.
11. ———— .. "Growth and fruiting of certain Ascomycetous fungi," *Ann. Bot. (N.S.)*, 1946, **10**, 185–94.
12. Herrick, J. A. .. "The carbon and nitrogen metabolism of *Stereum gausapatum*," *Obeo. J. Sci.*, 1940, **40**, 123–24.
13. Mandels, G. R. .. "Metabolism of sucrose and related oligosaccharides by spores of fungus *Myrothecium verrucaria*," *Plant Physiol.*, 1954, **29**, 18–26.

14. Margolin, A. S. .. *Ph.D. Thesis, University of West Virginia*, 1942.

15. Ranjan, S., Govindjee and Laloriya, M. M. "Chromatographic studies on amino acid metabolism of healthy and diseased leaves of *Croton sparsiflorus*," *Proc. Nat. Inst. Sci.*, 1955, **21**, 42–47.

16. Smith, V. M. .. "On mechanism of enzyme action by *Marasmius chordalis*," *Arch. Biochem.*, 1949, **23**, 446–72.

17. Tandon, R. N. and Bilgrami, K. S. "Effect of some nitrogen compounds on growth and sporulation of *Phyllosticta cycadina*," *Proc. Nat. Acad. Sci., (India)*, 1954, **24 B**, 191–96.

18. Wolf, F. T. .. "Utilization of carbon and nitrogen compounds by *Ustilago zeæ*," *Mycologia*, 1953, **45**, 516–22.

EXCITATION, INHIBITION, CONTRACTION, ACTION POTENTIALS AND CONDUCTION IN UNSTRIATED MUSCLE IN THE ABSENCE OF ANY ELECTROLYTE IN THE EXTERNAL MEDIUM

By Inderjit Singh, F.A.Sc. and A. K. Acharya

(From the Department of Physiology, Medical College, Agra)

Received September 14, 1957

Singh and Singh (1943) and Singh (1944) found that the stomach muscle of the frog, *Rana tigrina*, had the remarkable property that it remained irritable in hypotonic solution of sucrose for over 24 hours. The muscle gives powerful mechanical responses, accompanied by action potentials (Singh, 1957; Singh and Bhatt, 1957). It might be argued that these responses were due to sodium retained in the interspaces. This possibility was excluded by the observations which show that when the muscle has acclimatised to sucrose, the action of sodium chloride becomes inhibitory. After the muscle has remained in frequently renewed sucrose solution for about 3–4 hours, and when it is contracting strongly, sodium chloride inhibits the mechanical and electrical responses. Chemical analyses show that these responses appear when the interspaces are practically free of sodium (Singh and Acharya, 1957); the muscle is acclimatised to sucrose under these conditions. Potassium estimations show that the mechanical response is related not to extracellular sodium, but to intracellular potassium. In the present research, the inhibitory action of sodium and other ions and drugs has been further investigated.

Material and Methods

These experiments were performed on transverse pieces of the stomach muscle of the frog, *Rana tigrina*. The muscle was stimulated as described in previous papers. The experiments were performed at 30–32° C.; at this temperature the acclimatisation to sucrose (0·112 M). takes about 2–3 hours, the muscle gives large spontaneous contractions when acclimatised to sucrose, and the inhibitory action of ions is best obtained. Action potentials were recorded by a mechanical oscillograph.

Results

The action of sodium.—When the muscle has acclimatised to hypotonic solution of sucrose, it becomes quite sensitive to the action of sodium;

0·001 M NaCl produces complete inhibition which may last for 2–3 hours (Fig. 1 *a*, *b*). There may be little rise of tone here and there, but the inhibition of the spontaneous contractions is complete; 0·0005 M NaCl produces

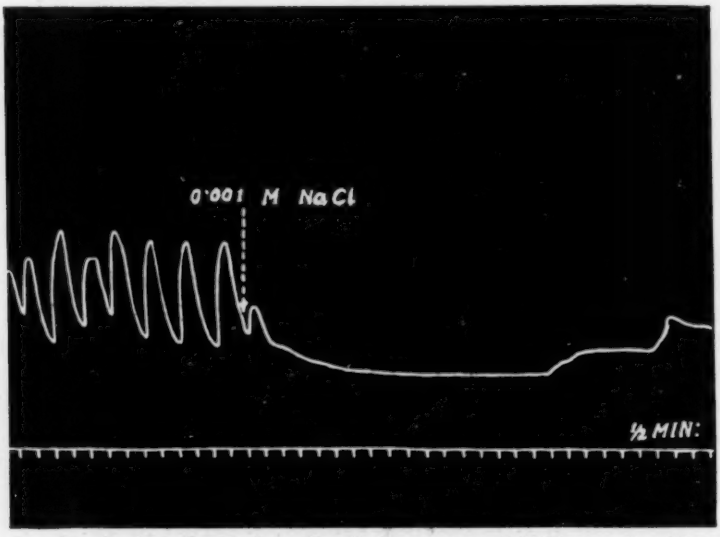


FIG. 1 *a*. Frog's stomach muscle acclimatised to sucrose. Inhibition produced by sodium chloride.

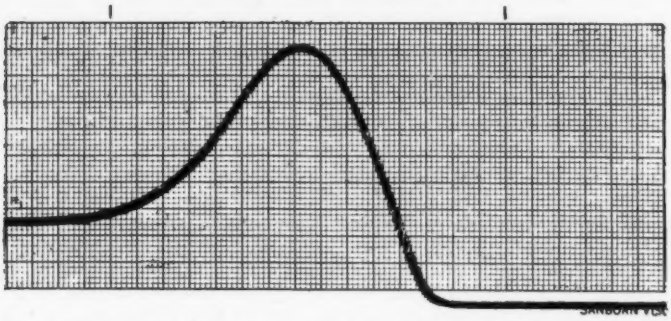


FIG. 1 *b*. Frog's stomach muscle. Action potential in sucrose, which is abolished by sodium.

partial inhibition. Higher concentrations of sodium chloride also produce inhibition. In sucrose solution diphasic action potentials are produced which are abolished by sodium. This shows that conduction of action potential can occur in the absence of any electrolyte in the external medium.

The action of magnesium.—The sucrose acclimatised muscle is also very sensitive to magnesium, 0·0007 M $MgCl_2$ producing complete inhibition like sodium chloride (Fig. 2). Higher concentrations also produce inhibition.

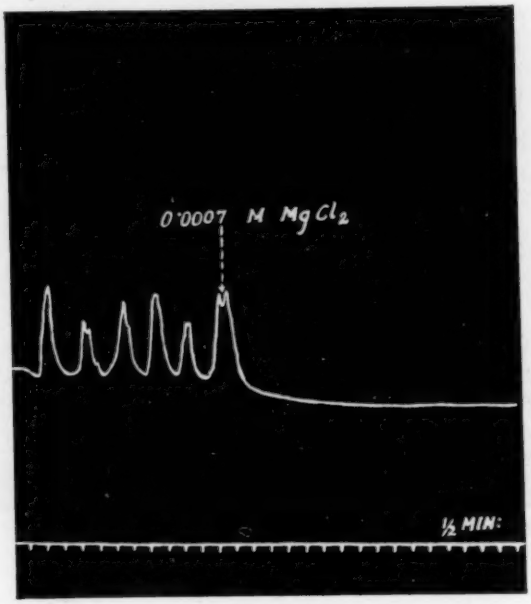


FIG. 2. Frog's stomach muscle acclimatised to sucrose. Inhibition produced by magnesium chloride.

Effect of calcium.—The action of calcium is different from that of sodium or magnesium. Small concentrations (0·007 M $CaCl_2$), produce increase in tone, as a result of which the spontaneous contractions become smaller and may be suppressed altogether. Calcium, thus, has an entirely opposite action in sucrose acclimatised muscle, compared to its action on muscle immersed in saline; when in saline, calcium promotes excitability and the spontaneous contractions. With increasing concentration of calcium, mixed effects are obtained. There is inhibition of tone as well as spontaneous contractions, alternating with increase of both, ultimately ending with increase of tone and suppression of the spontaneous contractions (Fig. 3). Thus, unlike its action in saline, the presence of calcium is not conducive to prolonged excitability of the sucrose acclimatised muscle. Calcium is thus required in the saline to antagonise some action of sodium.

Effect of barium and strontium.—The effects of these divalent ions are similar to those of large concentrations of calcium. In large concentrations,

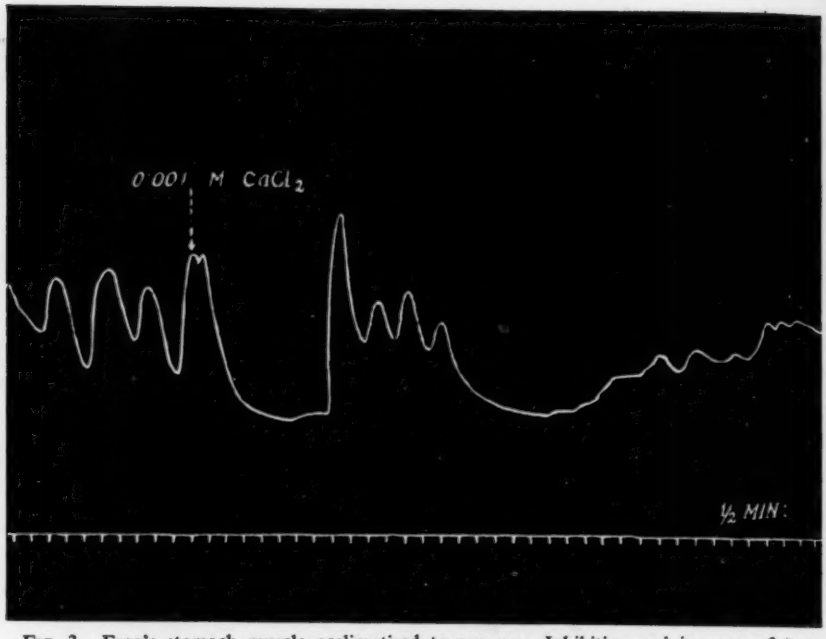


FIG. 3. Frog's stomach muscle acclimatised to sucrose. Inhibition and increase of tone produced by calcium.

0·02 M, strontium produces temporary inhibition. Alternately, as with calcium, tone increases, and the muscle passes into contracture. Barium does not produce inhibition, but increases tone in large concentrations (0·07 M; Fig. 4).

Effect of potassium.—In large concentrations, potassium produces inhibition; complete inhibition is produced by 20–40 times the concentration of sodium required to produce the same effect (Fig. 5). In concentrations similar to those of potassium, ammonium produces inhibition which lasts for a short time, the muscle may recover in about 10 minutes and begin to contract spontaneously.

Effect of adrenaline.—Sucrose acclimatised muscle is very sensitive to adrenaline, complete inhibition being produced by concentrations of 1 in 10^7 to 1 in 10^6 (Fig. 6). Muscle in saline is also similarly sensitive. This shows that inhibition can occur in the absence of any electrolyte in the external medium.

Effect of acetylcholine.—Frog's stomach muscle is not normally very sensitive to acetylcholine, contraction being produced by concentrations of

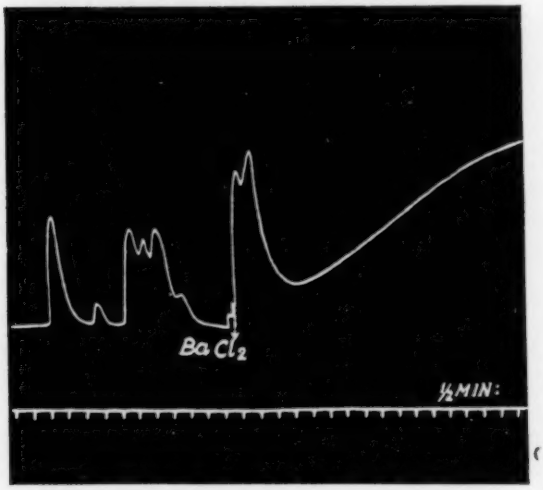


FIG. 4. Frog's stomach muscle acclimatised to sucrose. Contraction produced by barium (0·07 M $BaCl_2$).

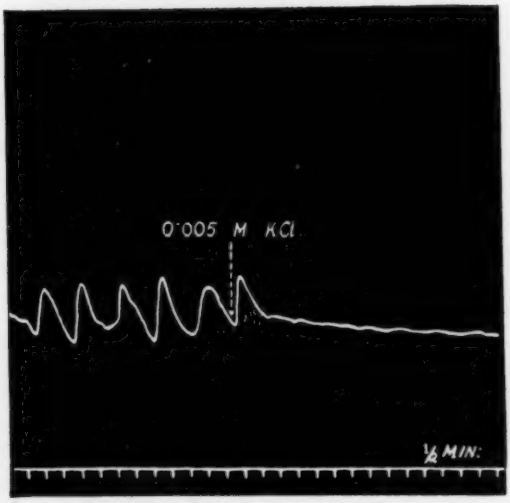


FIG. 5. Frog's stomach muscle acclimatised to sucrose. Inhibition produced by potassium.

1 in 10^5 to 1 in 10^4. For sucrose acclimatised muscle, similar concentrations are required. It causes contraction and increase of tone (Fig. 7).

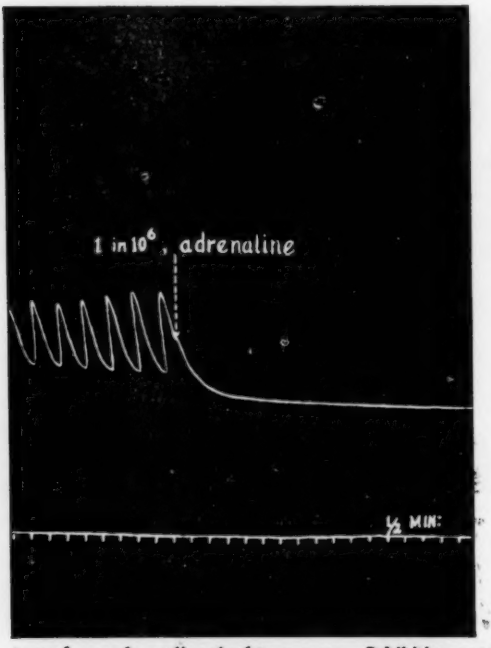


FIG. 6. Frog's stomach muscle acclimatised to sucrose. Inhibition produced by adrenaline.

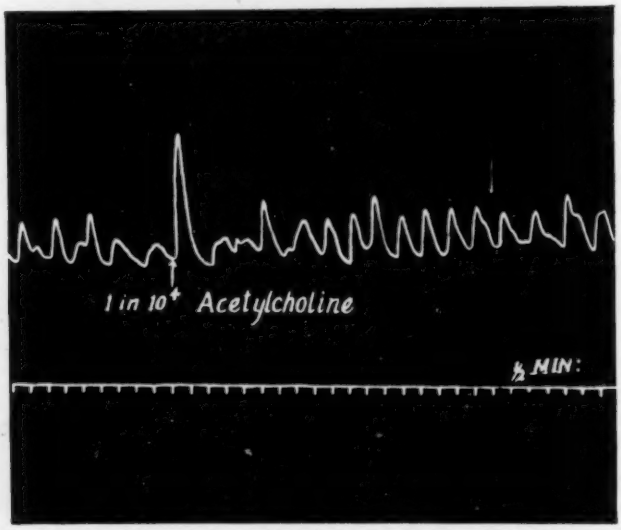


FIG. 7. Frog's stomach muscle acclimatised to sucrose. Contraction produced by acetylcholine.

Effect of anions.—The effect of other sodium salts was also studied. Sodium bromide, sodium nitrate, sodium iodide, sodium thiocyanate produce inhibition in large concentrations only (0·04 M upwards).

Discussion

When the muscle has acclimatised to sucrose, it becomes very sensitive to sodium chloride and magnesium chloride. Other ions cause inhibition or contraction in comparatively very large concentrations, and are of no interest at present. The significance of the inhibitory effect of magnesium is not understood.

The inhibitory effect of sodium conclusively shows that it is not necessary for excitability and action potentials. On the contrary it appears to be undesirable, the more completely it is removed from the interspaces, the better for the sucrose acclimatised muscle. Calcium thus appears to exert a baneful effect on this preparation; otherwise the muscle behaves in a normal fashion being just as responsive to adrenaline and acetylcholine. These experiments show that excitation, inhibition, contraction and relaxation, production of action potentials and their conduction can occur in the absence of any electrolyte in the external medium.

Summary

1. The inhibitory effect of ions and drugs on the sucrose acclimatised muscle has been studied.

2. Such a muscle is very sensitive to the inhibitory effect of sodium and magnesium. Sodium inhibits the diphasic action potentials.

3. Calcium causes a tonic contraction in small, and inhibition in large concentrations; the latter ultimately cause tonic contraction.

4. Barium produces a tonic contraction in large concentrations.

5. Strontium at first produces inhibition and then contraction in large concentrations.

6. Ammonium produces inhibition in large concentrations, which lasts for a few minutes, the muscle beginning to contract spontaneously.

7. Potassium causes inhibition in large concentrations.

8. Sodium bromide, nitrate iodide, thiocyanate produce inhibition in large concentrations.

9. The muscle is very sensitive to adrenaline, which produces inhibition.

10. Acetylcholine causes contraction.

REFERENCES

Singh, I. .. *Curr. Sci.*, 1944, **10**, 251.

——— .. *Presidential Address, Physiology Section, 44th Ind. Sci. Congress, Calcutta*, 1957

Singh, S. I. and Singh, I. .. *Proc. Ind. Acad. Sci.*, 1943, **18**, 58.

Singh, I and Bhatt, J. V. .. *Ibid.*, 1957, **65**, 64.

——— and Acharya, A. K. .. *Ibid.*, 1957.

947–57. Printed at The Bangalore Press, Bangalore City, by C. Vasudeva Rao, Superintendent. and Published by The Indian Academy of Sciences, Bangalore.

THERMAL FIELD AND OCEANIC CIRCULATION ALONG THE EAST COAST OF INDIA

By A. A. Ramasastry* and C. Balaramamurty

(*Andhra University, Waltair*)

Received 20 April, 1957

(Communicated by Dr. N. K. Panikkar, F.A.SC.)

Abstract

From the bathythermographic data, collected during the first 19 cruises of the Andhra University during the period October 1952 to April 1953 along the east coast of India, thermal fields at surface, 40, 100, 200 and 400 feet levels have been constructed for the three seasons, *viz.*, Post-Monsoon, Winter and Summer seasons. The present study is confined to the coastal region between latitudes 13° N. to 18° N. and longitudes 80° E. to 84° E. In addition to this area, during winter season, the study has been extended to an area over the *Swatch of No Ground.* From the above, circulation has been deduced and the current directions have been entered on the charts. The limitations and the validity of the method in entering the current directions is discussed and further it is shown, from a consideration of the current boundaries, that the method yields a fairly good representation of the oceanic circulation at various depths. Seasonal variation in the thermal structure and the associated circulation is presented.

In the Post-Monsoon and Hot Weather seasons the current pattern indicates turbulence in the surface layers as a consequence of sinking and upwelling. Greater turbulence occurs off Visakhapatnam than off Madras. At a level at which thermocline forms, currents are opposing to those found above and below that level. A nearly isothermal water mass which forms over the continental shelf during the Hot Weather season appears as a semi-permanent feature, keeping its position due to the existing north-easterly current.

1. Introduction

LaFond (1954), LaFond and Borreswara Rao (in print) and Ramasastry (1954) have made extensive studies on the temperatures of the Bay of Bengal and have indicated the possible currents in the Bay. But they have not constructed any charts showing the currents in the Bay. In the present investi-

* Present address: Central Marine Fisheries Research Station, Ernakulam.

gation charts showing the directions of currents at various depths have been presented, each set to represent a particular season.

2. TREATMENT OF DATA

Numerous bathythermographic data along the east coast of India are available from October 1952 to April 1953, the data having been collected by the Andhra University personnel as a part of the research programme for that year. Part of those observations which lie between latitudes 13° N. and 18° N. and longitudes 80° E. and 84° E. have been utilised in the present investigation and in addition observations in December 1952 over one more area, the *Swatch of No Ground*, the submarine canyon, at the mouths of the Ganges and the Brahmaputra, have been utilised. The total period of observations has been divided into three seasons, *viz.*, the '*Retreating South-west Monsoon season*' or the '*Post-Monsoon season*' (October and November), the '*North east Monsoon season*' (December–February) and the '*Hot Weather season*' (March–April). The positions of some of the stations falling into each of these seasons are shown as dots in the respective surface charts. For each of these seasons isotherms at 1° F. intervals at surface, 40, 100, 200 and 400 feet depth levels have been constructed. From the thermal fields so obtained the direction of currents has been entered, bearing in mind the following points.

From the dynamics of ocean currents one derives an important relation between the current and the distribution of mass: "*The current flows in such a direction that the water of low density is on the right-hand side of the current and the water of high density on the left-hand side*," this being the case for the northern hemisphere. But at the surface the variation of temperature depends on many factors such as the incoming solar radiation, the time of the day and the season of the year, the difference between the air temperature and the sea surface temperature, the amounts of precipitation, condensation and evaporation and on the surface winds, etc. Advective processes in the sea and the atmosphere near the sea suface will result in temperature changes. Hence the thermal fields of the surface or a few feet below the sea surface for any season would be complicated and if one derives currents at the surface, purely from the thermal field, the current pattern would be rather complicated.

Another important factor to be considered while drawing currents is the distribution of salinity, as the density of sea-water depends on salinity as well. Surface salinity in the Bay of Bengal shows a considerable annual variation due to large amounts of dilution from the main rivers, the Ganges, the Brahmaputra, the Godavari and the Krishna. Sewell (1929) has studied

the distribution of surface salinity in the Bay. The authors in a separate paper have considered the Sigma-t field at the surface for drawing the surface currents wherein certain departures from the pattern obtained by the present method are discussed.

For plotting the currents at the surface and in the mixed layer, the distribution of wind is also important and hence its effect is also considered. Hence on the surface charts of the thermal field and the associated currents, observed winds have been entered in Beaufort scale at some of the stations. Depending upon the duration, intensity and fetch of the wind the depth of the mixed layer will be greatly affected resulting in a rearrangement of mass, especially in stable waters. In such developments of top homogeneous layers wind currents will be impeded.

Upwelling and sinking along the coast result from the semi-permanent wind systems such as the monsoon winds along the east coast of India. LaFond (1954) has examined upwelling and sinking along this coast from March to May and from September to November associated with the North-east and South-west monsoons respectively. When upwelling takes place the surface flow will be from regions of low temperature to the regions of high temperature and the sub-surface flow will be in the opposite direction. The reverse will be the case with sinking.

If tongue-like distributions of temperature is found currents can be entered bearing in mind that water of high temperature should be on the right of the current. In such a case the current pattern would be almost along the axis of the tongue. But in general this does not give true picture because of the negligence of the frictional forces and it will be more so at the surface where the temperature depends on many external factors. Sverdrup and Fleming (Oceans, 1946) have found that drift bottle experiments have confirmed the results in a certain case where the flow pattern has been derived on the assumption that the stream lines tend to follow the contour lines of the tongues.

Finally at the coast, currents follow the coast-line. For the subsurface currents the appropriate contour line acts as the coast-line. Ekman also found that a current will be deflected *cum sole* while entering shallower water and *contra solem* while entering deeper water and that these deflections are independent of the absolute depth of the bottom. Considering all these facts current directions are entered on the charts.

3. Discussion of the Thermal Field and the Associated Currents

The Retreating South-west Monsoon Season (October–November).—With the retreat of the South-west Monsoon from India, the centre of low pressure

has shifted into the centre of the Bay of Bengal and the winds are mainly north-easterly over the region under investigation. With the wind pattern, both at the surface and at 40 feet depth, the currents are mainly directed south-westerly and flow along the coast.

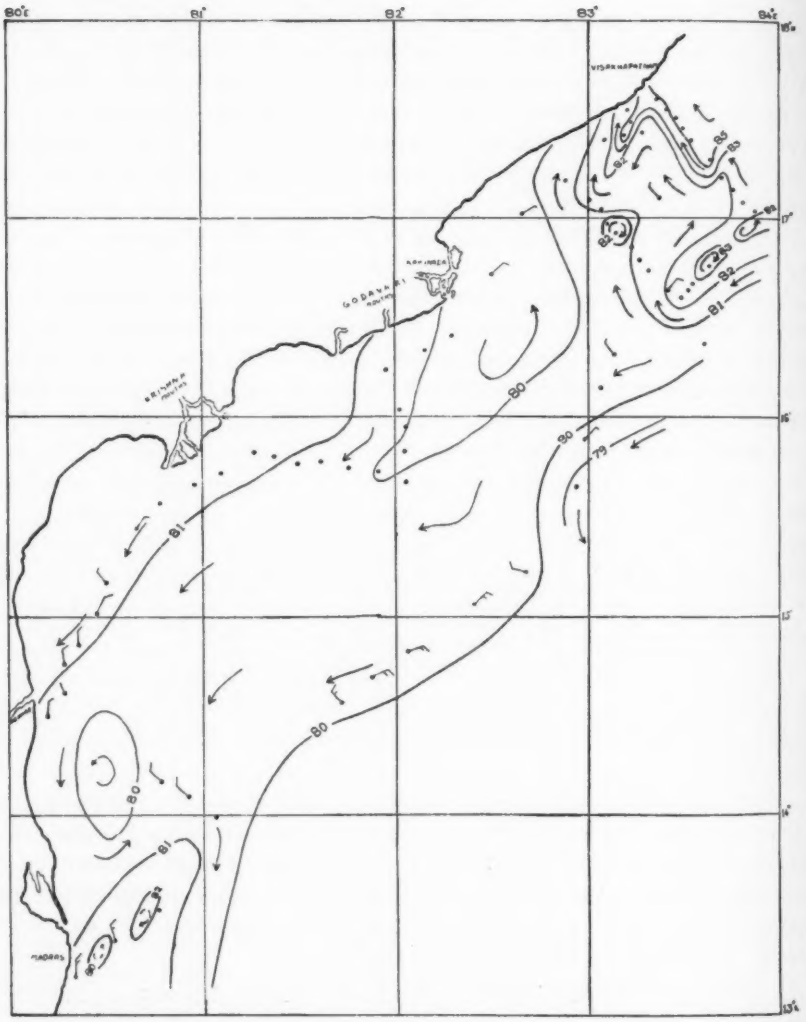


FIG. 1. Thermal field and the associated currents at the sea surface during the Post-Monsoon season along the east coast of India in the region between 13° N. to 18° N. and 80° E. to 84° E.

The temperature gradually increases with increasing distance from the coast. Except near the Godavari and the Krishna, south of Vishakapatnam isotherms at both these levels run almost parallel to the coast. A tongue of low temperature is however found off the mouths of the Godavari at the surface (Fig. 1) and also a zone of low temperature off the Pennar. The low temperature zones are characteristic of the river discharges as the run-off from the rivers is generally of lower temperature because of the difference of temperatures between the land and water surfaces. Off Viskahapatnam, however, the isotherms run almost perpendicular to the coast with decreasing temperatures with distance from the coast. Also the temperature decreases towards south. The general trend of low temperatures in the south may partly be attributed to the shift of the rainy season to the Madras coast.

At the surface a coastal current flows towards south below latitude 16° N. Off the Godavari, due to the tongue of low temperature, a current flows almost in the opposite direction. Due to sinking along this coast in this season the currents should be expected to run from lower temperature to higher temperature side. Such a feature is also clear off the Godavari and off Visakhapatnam. At the latter place a strong current is seen to flow towards the coast at the surface and also at 40 feet depth (Fig. 2). The cellular structure of the complicated pattern of the isotherms and the associated currents off Visakhapatnam, both at the surface and at 40 feet, indicate the amount of turbulence present. The variable winds accompanied by the slow retreat of the monsoon and followed by sinking will generally result in thorough mixing of the upper layers and the formation of eddies.

About 60 miles off Visakhapatnam two rotating currents in opposite directions are noticed at the surface and at 40 feet, while the two merged into one counterclockwise rotation at the 100 feet level showing reduced intensity of turbulence at 100 feet. Only at surface, off Madras, a small zone of high temperature characterised by two opposing currents, purely of thermal origin, are found. These currents (marked in discontinuous arrows) should not be considered too rigidly as the salinity here is high compared to that in the farther north, where much dilution takes place. Considering these two factors the general character of the surface currents should be south-westerly.

Immediately after the run-off from the Godavari at the Sacramento (off Kakinada) in the tongue of low temperature a counterclockwise rotation occurs at the surface while at 40 feet depth it splits up into two oppositely rotating currents, the opposing feature being centered at 16° 20′ N. and 82° 20′ E. which is completely absent in deeper waters. This might be due

to the lesser dilution at this place as the fresh-water of the Godavari is taken up northwards by the surface current.

FIG. 2. Thermal field and the associated currents at 40 ft. depth level during the Post-Monsoon season along the east coast of India in the region between 13° N. to 18° N. and 80° E. to 84° E.

At 100 feet (Fig. 3) the temperature field is such that the temperature increases with increasing distance from the shore. As sinking inshore is prominent, at subsurface depths currents should be directed from higher temperatures to the lower temperature side which means a reversal of currents

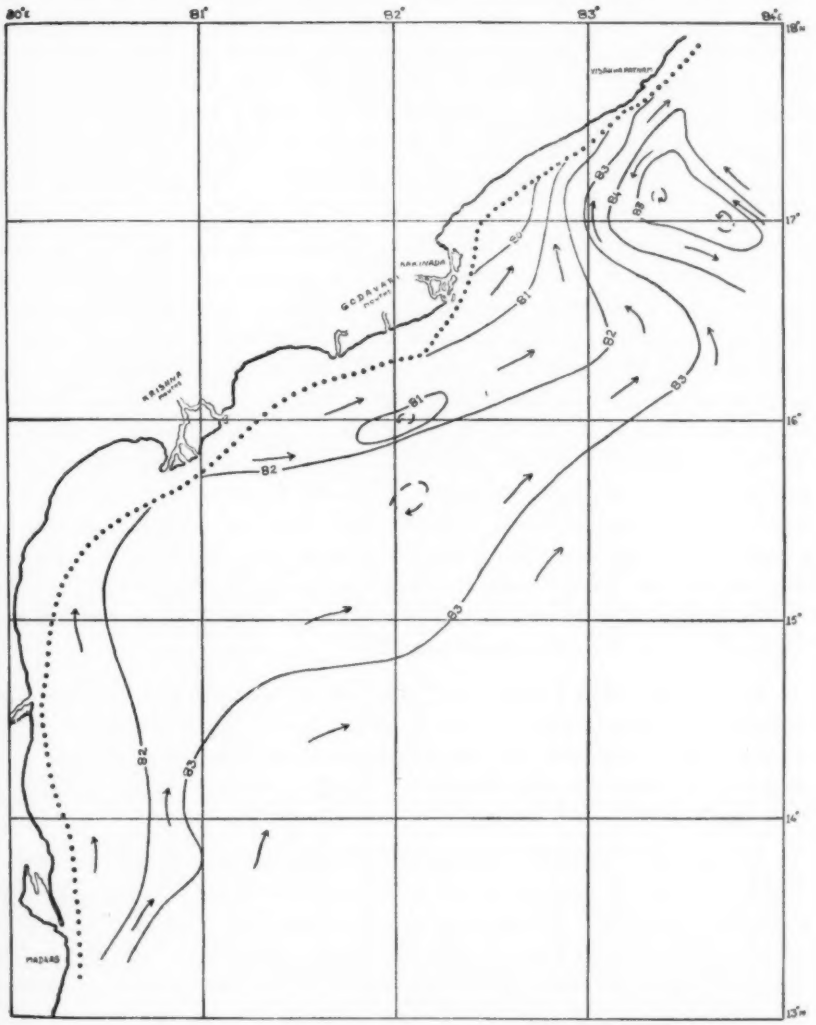


FIG. 3. Thermal field and the associated currents at 100 ft. depth level during the Post-Monsoon season along the east coast of India in the region between 13° N. to 18° N. and 80° E. to 84° E.

as compared to those at the surface and at 40 feet depths. Such a feature is clearly noticed in the circulation along this coast at this depth. The development of thermocline increases in depth as one approaches the shore due to sinking and consequently the temperatures near shore will be lower. Consequently, currents at 100 feet alone are nearly in opposite direction to those above and below this depth. The tongue of low temperature, characteristic of the surface waters off the Godavari, has merged into the trough of low temperature which in general is found at this level along the entire coast. Further, turbulent mixing accompanied by sinking near Madras is almost eliminated. But off Visakhapatnam the eddy formation is still persisting at 100 feet indicating that sinking, even at this level, is more intense than farther south.

But at 200 feet (Fig. 4) the effects of sinking are completely absent even off Visakhapatnam. Upto 100 feet level the intensity of currents as indicated by the weak temperature field is very low whereas at 200 feet the intensity has increased with increasing intensity in the horizontal temperature gradients. Off the Godavari a subsurface counter current of low intensity is associated with a tongue-like distribution of higher temperatures in-shore. This, not only shows the continuity of the system at the place but also an increase in vertical stability at this level. The senior author has elsewhere shown that the vertical stability in this region was decreasing in the topmost layers with a depthwise increase thereafter. Except very near the coast the currents are more orderly and towards south-west the small eddy formation off Visakhapatnam, and off the Krishna and the Pennar can be respectively attributed to mixing followed by sinking and to dilution. Partly these may be associated with the bottom topography also.

At 400 feet (Fig. 5) level a few isotherms run parallel to the coast and the temperature increases with distance from the coast. The current is somewhat weak and follows the 400 feet line (shown as dotted line along the coast in the figure) in a south-westerly direction. Small deviations from the 400 feet line are associated with the effect of the topography.

Horizontally rotating systems off Visakhapatnam and off the Pennar at 400 feet, and at Madras coast at 200 feet, result due to the effect of the subsurface maximum temperature zone produced by sinking. Towards the middle of this season, due to winter cooling, at the surface; and by the sinking of the surface-waters inshore, the bathythermograms show inversions of temperature at subsurface depths resulting in a zone of maximum temperature round about 200 feet level, which is apt to reduce the vertical stability. As has been pointed out already, stability decreases in the top layers and shows

an increase at these depths. This is because of much dilution of the surface waters. However, an increase in stability at these levels impedes vertical

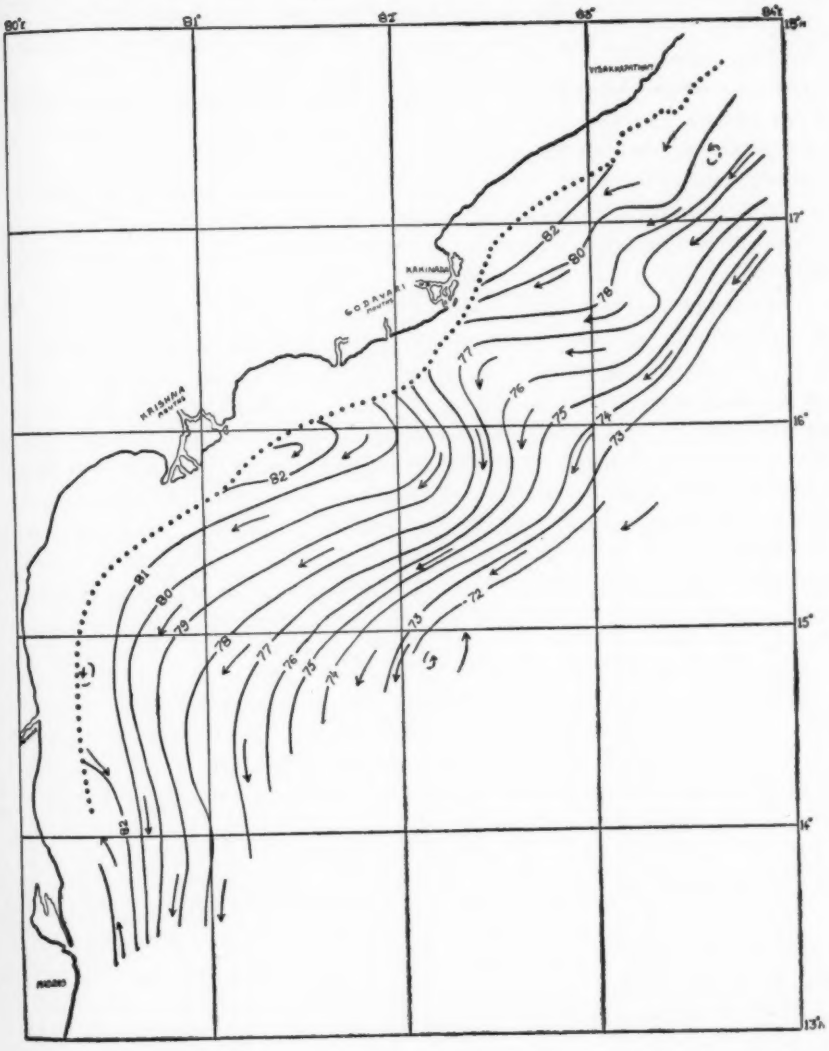


FIG. 4. Thermal field and the associated currents at 200 ft. depth level during the Post-Monsoon season along the east coast of India in the region between 13° N. to 18° N. and 80° E. to 84° E.

movement. Hence as a consequence of the subsurface temperature inversions, in specified regions, clockwise rotations have formed.

The North-east Monsoon Season (*December–February*).—During this season observations were made north of the Godavari upto the mouths of

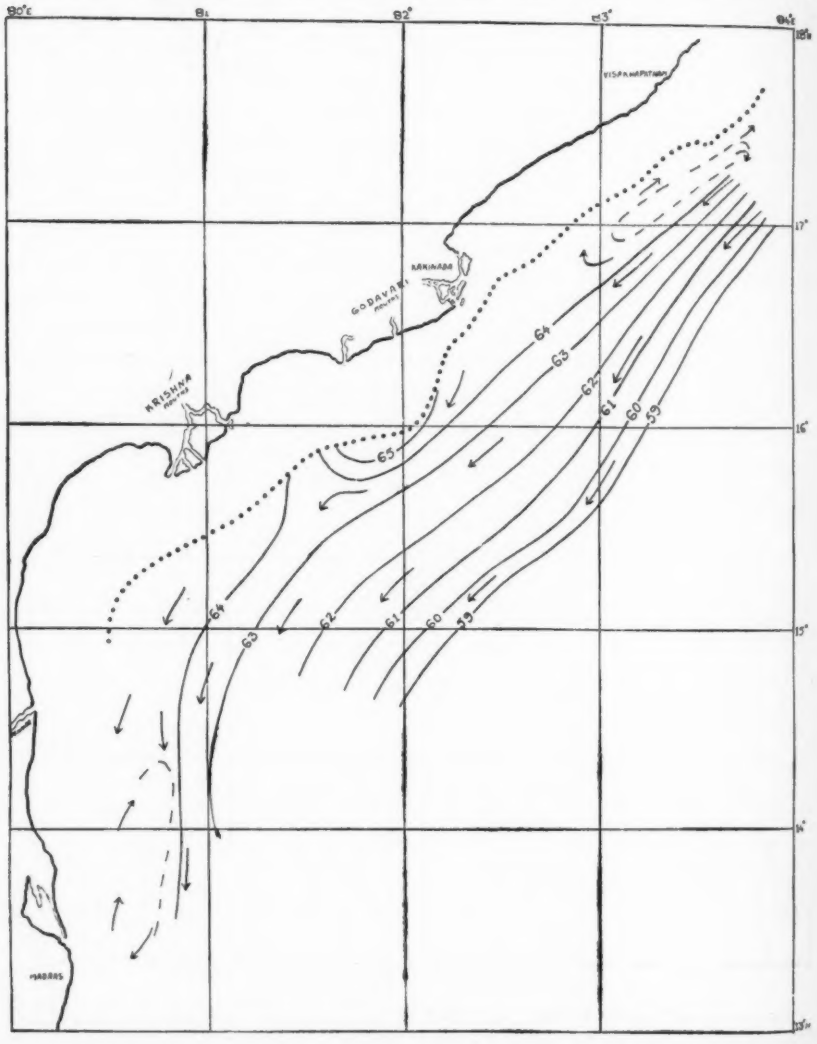


FIG. 5. Thermal field and the associated currents at 400 ft. depth level during the Post-Monsoon season along the east coast of India in the region between 31° N. to 18° N. and 80° E. to 84° E.

the Ganges. Figures 6–10 and 11–15 represent the thermal field and the associated currents at the coastal region from the Godavari to Visakhapatnam and at the head of the Bay of Bengal respectively.

During this season the surface is cooled by about 2° F. than in the previous season. Due to the shift of the rainy season to the extreme south of peninsular India and the extreme fair and cold weather conditions all over India the run-off from the rivers is very much reduced in intensity with a consequent rise in salinity of the surface layers. With the increase in salinity and lowering of temperature the density of the upper layers becomes much and vertical convection currents set in. Hence, except early in this season, the subsurface zone of maximum temperature is eliminated (LaFond and Borreswara Rao, 1954; Ramasastry, 1954). The depth of thermocline off Visakhapatnam is greater than that off Kakinada. The depth of the thermocline is greater near the coast than in the offshore waters. Sinking is almost coming to an end early in this season when the phenomenon is confined entirely near the coast. As the season advances sinking reduces in intensity and towards the end the phenomenon is absent. Winds in the region under consideration are very weak and to a certain extent variable in direction early in this season. Owing to all these changes the thermal field at the surface is very complicated (Fig. 6) while at 40 or 100 feet levels it is more orderly. In general temperatures at the surface increase with increasing distance from the shore and the thermal field is poorly defined.

Turbulent mixing of waters, followed by the absence of sinking over the middle of the continental shelf, is confined to the coastal region where two small rotating systems off Visakhapatnam and one off the Sacramento are formed. These are still manifested upto a depth of 200 feet. The main trend of the current in general is towards north-east at the surface (Fig. 6).

At 40 feet (Fig. 7) where the external influencing factors of temperature are less predominant, the clockwise rotation south of Visakhapatnam has gained in dimensions and has formed into a big rotating system. Another such system with still more intensity is found off Visakhapatnam. The two are separated by a small region of low temperatures which at 100 feet level has developed into a tongue of low temperature. Unlike in the previous figure, at 100 feet level (Fig. 8) this tongue of low temperature separates two current systems which are nearly opposing one another. The southerly one is in the anticlockwise direction while the northern system is of variable currents and has a general tendency to rotate in the clockwise direction. The general trend of the offshore current is north-easterly even upto this level.

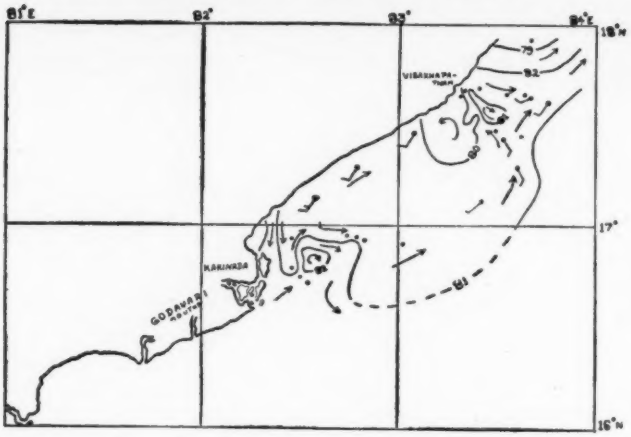


FIG. 6. Thermal field and the associated currents at the sea surface during the North-east Monsoon season along the east coast of India in the region between 16° N. to 18° N. and 81° E. to 84° E.

The apparent reverse direction at 100 feet level near the Godavari is formed because of the development of thermocline at shallower depths in this region than off Visakhapatnam, where it is formed nearly at 200 feet. This is clearly brought out by an opposing current off Visakhapatnam noticed at 400 feet level. At the transition depth of formation of the thermocline currents are relatively weak and variable in direction. The effect of the res-

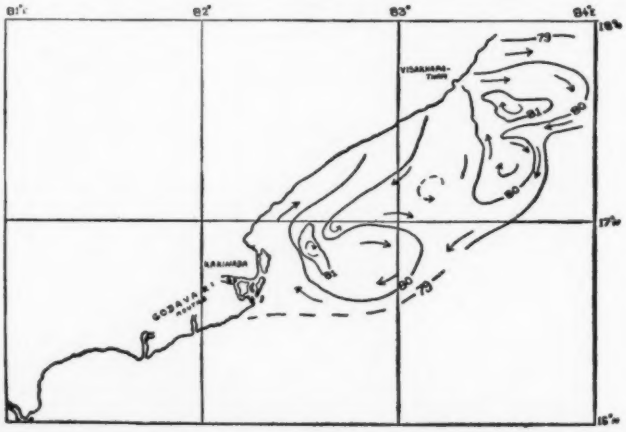


FIG. 7. Thermal field and the associated currents at 40 ft. depth level during the North-east Monsoon season along the east coast of India in the region between 16° N. to 18° N. and 81° E. to 84° E.

pective contour lines and the bottom topography at 200 and 400 feet plays an important role in producing deviations in the current directions. This is the transition season between the period of sinking and that of upwelling. Hence while examining the coastal currents one will find a complicated pattern.

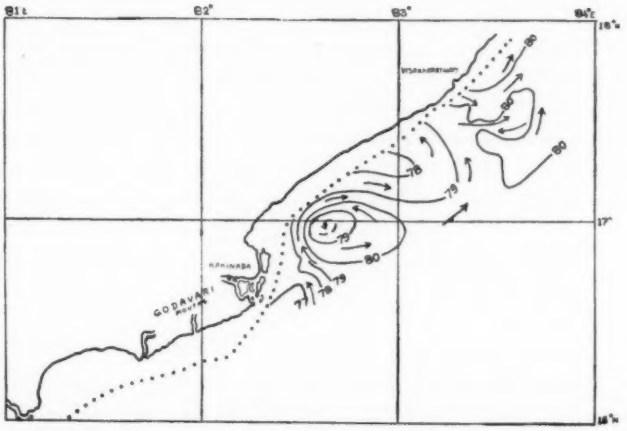


FIG. 8. Thermal field and the associated currents at the 100 ft. depth level during the North-east Monsoon season along the east coast of India in the region between 16° N. to 18° N. and 81° E. to 84° E.

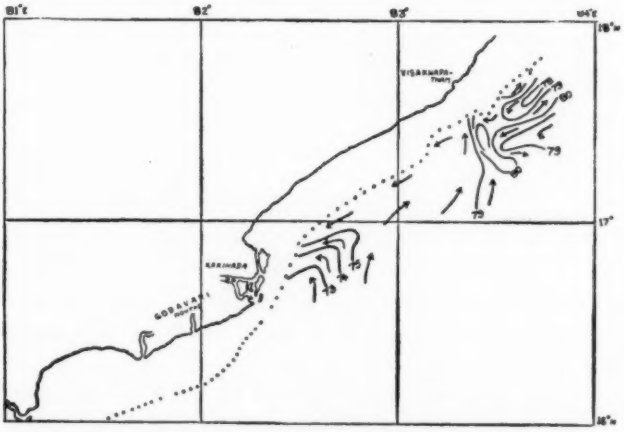


FIG. 9. Thermal field and the associated currents at the 200 ft. depth level during the North-east Monsoon season along the east coast of India in the region between 16° N. to 18° N. and 81° E. to 84° E.

It would be of interest to examine the currents along this coast during this season much more extensively and to a much larger distances from the coast.

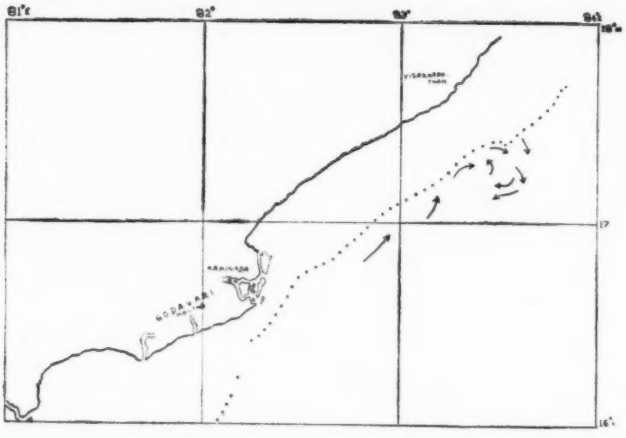


FIG. 10. Thermal field and the associated currents at the 400 ft. depth level during the North-east Monsoon season along the east coast of India in the region between 16° N. to 18° N. and 81° E. to 84° E.

Currents at the Head of the Bay of Bengal.—The influence of dilution due to the discharges of the Ganges and the Brahmaputra is reflected both in the

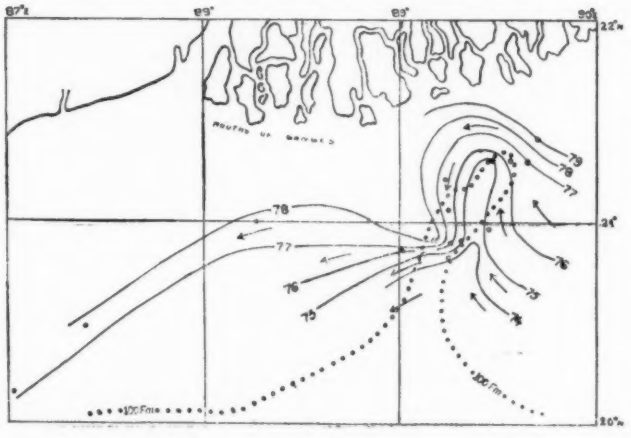


FIG. 11. Thermal field and the associated currents at the sea surface during the North-east Monsoon over the *Swatch of No Ground.*

thermal structure and the current pattern at least upto 100 feet level (Figs. 11–13). At the surface (Fig. 11) a tongue of low temperature extends from the south of the canyon (the canyon defined by the 100 fm. line, shown in dots in the figure) upto the northern extremity of the canyon. Another tongue of high temperature has formed to the south-west of the canyon. In general, along this coast upto the Mahanadi, temperatures decrease with increasing distance from the coast, whereas an increase in temperature is found off the Visakhapatnam coast. Hence the resulting currents oppose each other at these two localities forming an anticlockwise rotation in a region north of Visakhapatnam and south of the Mahanadi. Such an effect is prominent even upto a depth of 200 feet level (Fig. 14) as is seen by a comparison of the corresponding charts for this season. Due to the two tongue-like formations of temperature at the surface (Fig. 11) an anticlockwise rotation of currents is noticed over the *Swatch of No Ground.*

At 40 feet level (Fig. 12) the tongue of low temperature is surrounded by isotherms of high temperature. Extreme low temperatures are noticed

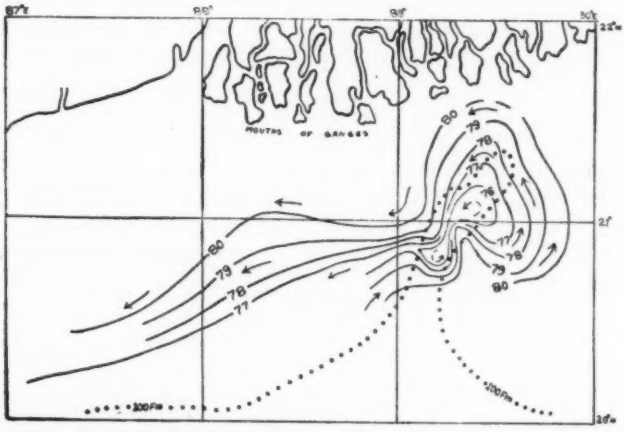


FIG. 12. Thermal field and the associated currents at 40 ft. depth level during the North-east Monsoon season over the *Swatch of No Ground.*

to the south of the canyon. Hence at this level a big clockwise rotation results. A reversal current is not found to any appreciable extent as an effect of the formation of the thermocline. A thermocline in fact did not develop in the top layers and instead a temperature inversion has been noticed upto 200 feet. The rise of temperature between surface and 200 feet is about 5° F. However over the *Swatch of No Ground* the depth of the thermocline is very great.

At surface and at 40 feet level the currents are mainly related to the river discharges while at still lower depths the effect of the bottom topography is felt. The diluted water flows towards the south or south-west where once again good mixing takes place in the eddy formation north of Visakhapatnam coast. Ramasastry (unpublished) studied the vertical stability of the sea-water upto 100 m. level in this region and found that the stability in general decreases with depth. At stations which lie exactly on the boundary of the *Swatch of No Ground*, quite unstable waters are noticed. As a matter of fact, due to higher temperatures near the coast and due to the high dilution one is to expect that the topmost waters to be lighter than those below. If this be the case the vertical stability must increase with increasing depth. But the contradicting observation can only be explained as due to turbulent mixing when the run-off of the rivers mix with the sea-water. This is partly produced by the presence of the submarine canyon. This latter conclusion is justified from the fact that good stability exists off the Godavari or off the Krishna. A recent observation of dilution from the Krishna has further strengthened this view. Photographs taken of the line of demarkation between the river water and the sea-water clearly showed the spread of the river water over the sea surface. Further there is a marked difference in salinity at the boundary surface.

As turbulent mixing at the *Swatch of No Ground* is not completed, a further clockwise gyrale has to form farther south. The intensity of mixing is much restricted above 100 feet level, as is seen from the close pack of the

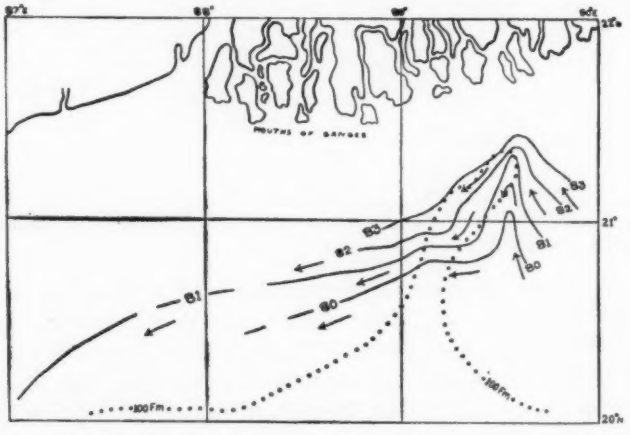


FIG. 13. Thermal field and the associated currents at 100 ft. depth level during the North-east Monsoon season over the *Swatch of No Ground*.

isotherms. At 100 feet level the spacing between the isotherms has increased, but a tongue of low temperature is extending over the *Swatch of No Ground* from the south. As a consequence of this a north-westerly current from the Burma coast has turned along this coast to form a south coastal current along the Indian coast.

At 200 and 400 feet levels (Figs. 14 and 15) the observations are relatively poor. At both these levels a current enters into the canyon and gets itself

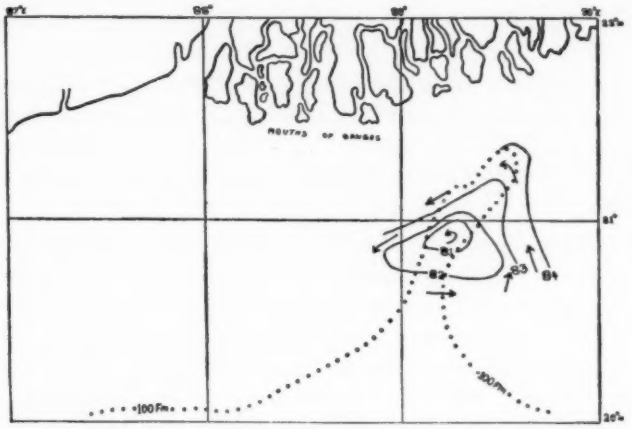


FIG. 14. Thermal field and the associated currents at 200 ft. depth level during the North-east Monsoon season over the *Swatch of No Ground*.

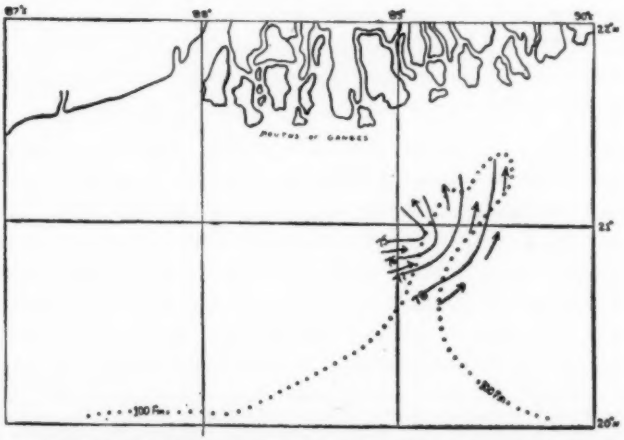


FIG. 15. Thermal field and the associated currents at 400 ft. depth level during the North-east Monsoon season over the *Swatch of No Ground*.

reflected and turns southwards. As a consequence of the formation of the thermocline below 300 feet a tongue of low temperature replaces the tongue of high temperature found in the surface chart. This results in currents in the opposite directions at this level.

The Hot Weather Season (*March and April*).—This season is characterised by intense heating and by comparatively weak winds of variable direction and towards the end of the season winds gain in strength and become more south-westerly as the monsoon begins to break from the southern parts of peninsular India. Due to north-easterly winds in the previous season surface water is carried away from the coast as a consequence of which subsurface water rises resulting in upwelling which gains intensity by the middle of this season. In a narrow zone over the continental shelf near the coast the upwelling is more intense resulting in low temperatures. Ramasastry (1954) has found that upwelling during April is more intense off Visakhapatnam than off Madras.

Off Visakhapatnam, at the surface (Fig. 16), the temperature near coast is 81° F. while over the continental shelf a region of high temperature is found. Here the isotherms are almost perpendicular to the coast. Further into the sea temperatures decrease from the zone of maximum temperature (84° F. to 81° F.). Similar features are noticed off the Godavari at 40 feet level (Fig. 17) while at the surface a low temperature zone is surrounded by isotherms of higher temperature (84° F.). The temperature in both the cases decreases towards east. But conditions off Madras are slightly different. There is a gradual increase in temperature (from about 80–82° F.) with increasing distance from the coast. The thermal structure at surface and 400 feet levels along the coast, from Visakhapatnam to the Godavari, is somewhat complicated and the associated currents generally show eddy formations. This is a consequence of turbulent mixing followed by upwelling.

At the surface and 40 feet levels the current pattern, very near the shore, exhibit a clockwise rotation off Visakhapatnam and north of the Godavari, and a counterclockwise rotation off south of the Godavari. The latter is due to the mixing of the Godavari waters. Well off into the sea the main current in this season is north-easterly. This is in conformity with Sewell's findings of the surface currents in this season which show a northerly current south of the Peninsula as entering the Bay and taking a turn following the coast towards north-east and forming two clockwise gyrales centered at 15° N. and 90° E. Eddy formation still persists even upto a depth of 200 feet.

At 100 and 200 feet levels (Figs. 18 and 19) off Visakhapatnam the coastal currents are nearly opposing one another showing that upwelling continues

from a depth of 100 feet level while at 200 feet level the intensity is further reduced resulting even in a counter-flow. Upto a depth of 400 feet the *East Indian Coastal Current* is north-easterly and temperature increases with distance away from the coast. The reason for a belt of low temperature

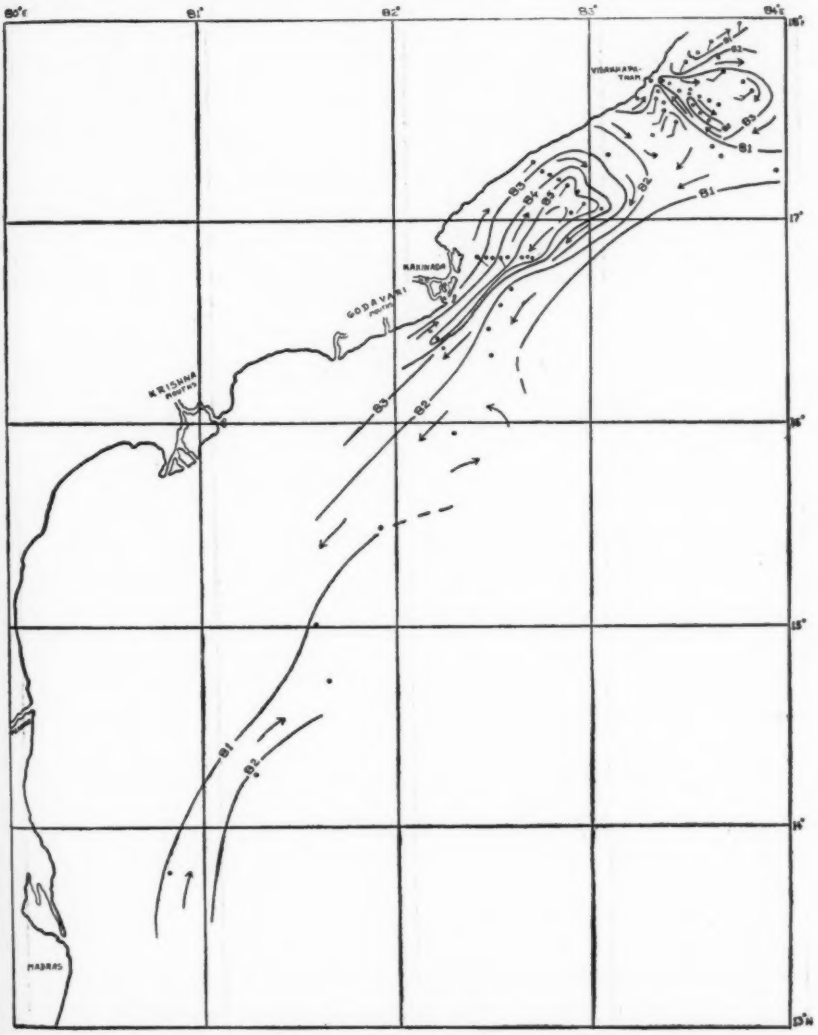


FIG. 16. Thermal field and the associated currents at the sea surface during the Hot Weather season along the east coast of India in the region between 13° N. to 18° N. and 80° E. to 84° E.

along the coast is the formation of the thermocline in the topmost layers of the sea as a consequence of upwelling.

While discussing the formation of a bottom watermass over the continental shelf characterised by zero gradients of temperature in the vertical,

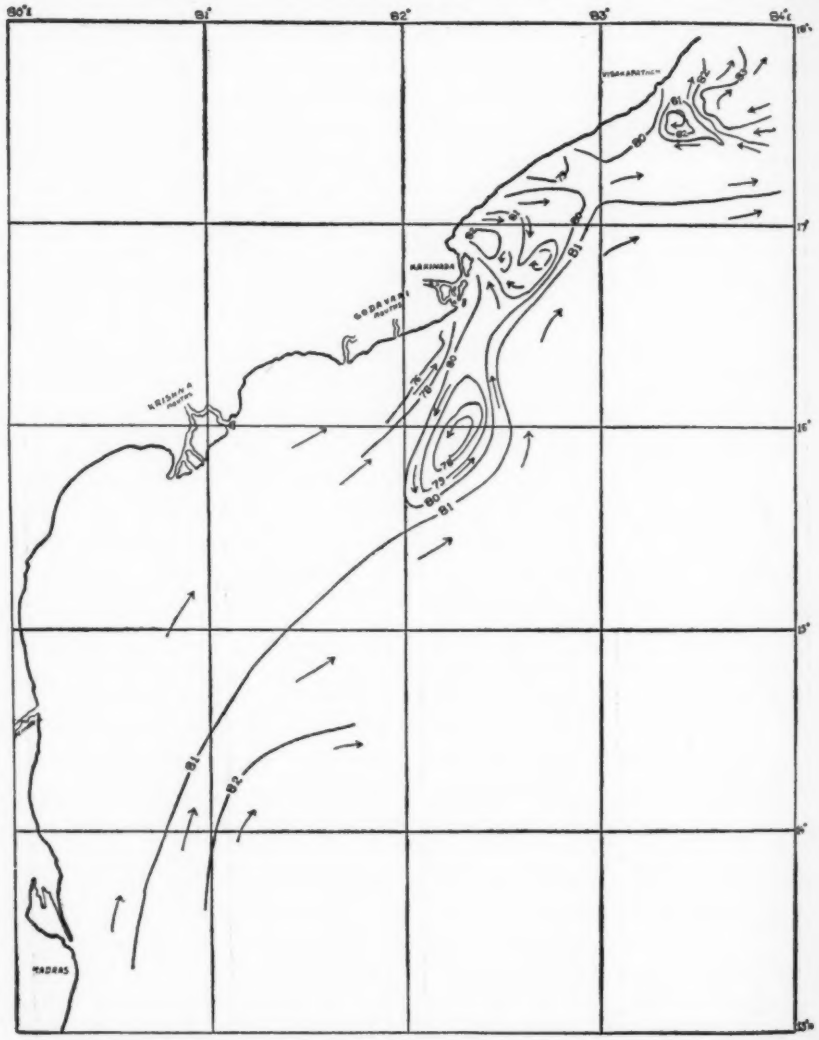


FIG. 17. Thermal field and the associated currents at 40 ft. depth level during the Hot Weather season along the east coast of India in the region between 13° N. to 18° N. and 80° E. to 84° E.

Ramasastry (unpublished) has indicated the possibility of turbulent and lateral mixing along this coast. Such features are confirmed by the present study off Visakhapatnam where large eddies are seen even upto a depth of 400 feet. Also it has been found that off the Godavari there exists this type of water

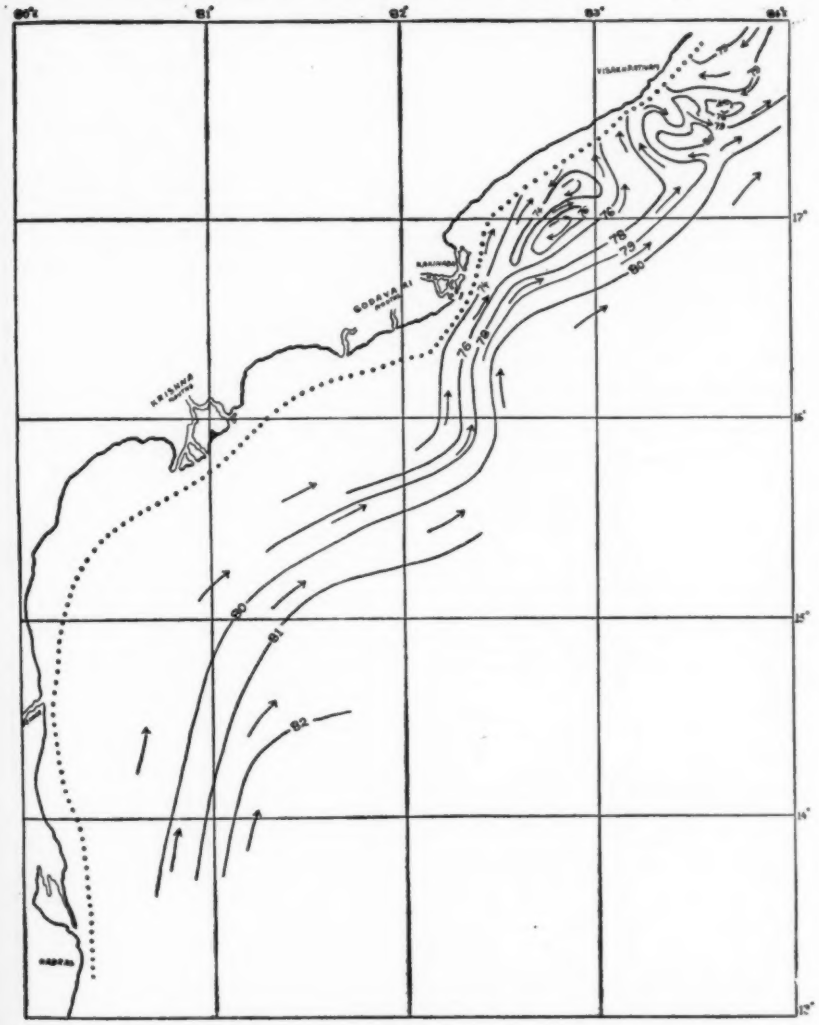


FIG. 18. Thermal field and the associated currents at 100 ft. depth level during the Hot Weather season along the east coast of India in the region between 13° N. to 18° N. and 80° E. to 84° E.

over the continental shelf. The bent of the *East Indian Coastal Current* towards east and north-east at 400 and 200 feet levels respectively keeps the position of the present watermass on the continental shelf for a fairly long period in this season. With the weakening of upwelling and the onset of

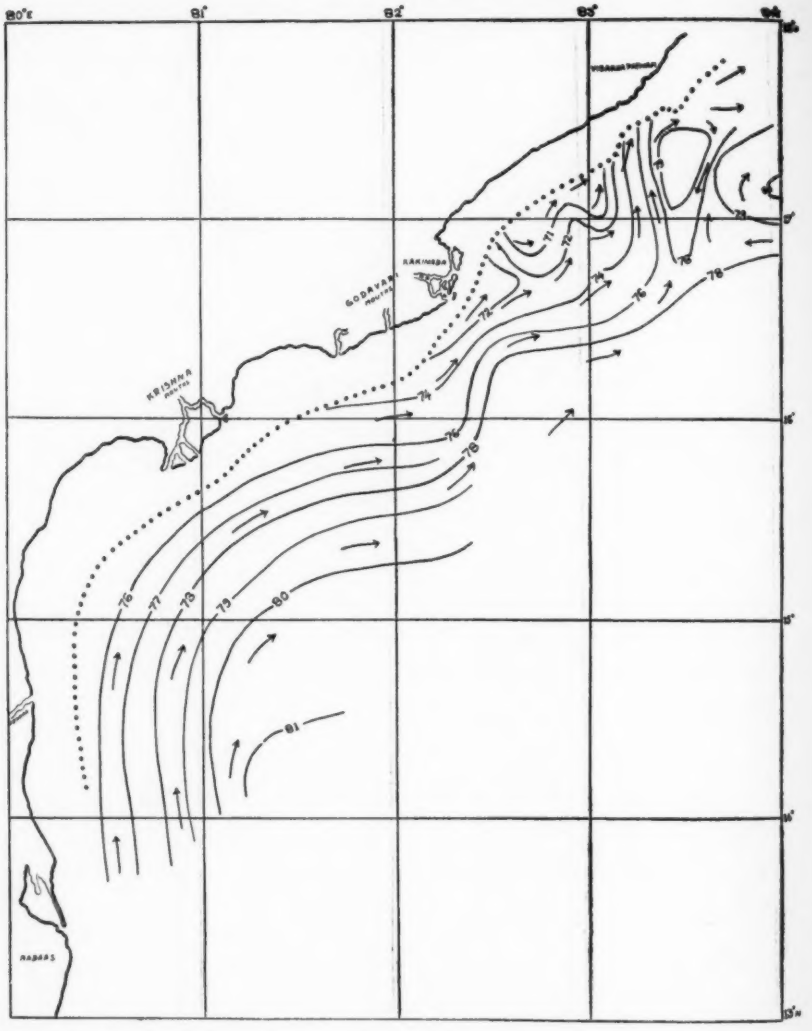


FIG. 19. Thermal field and the associated currents at 200 ft. depth level during the Hot Weather season along the east coast of India in the region between 13° N. to 18° N. and 80° E. to 84.° E.

monsoon, this feature gradually disappears. Further the water over the continental shelf is carried north-east by the *North Equatorial Current* of the Indian Ocean which forms a north-easterly flowing coastal current along this

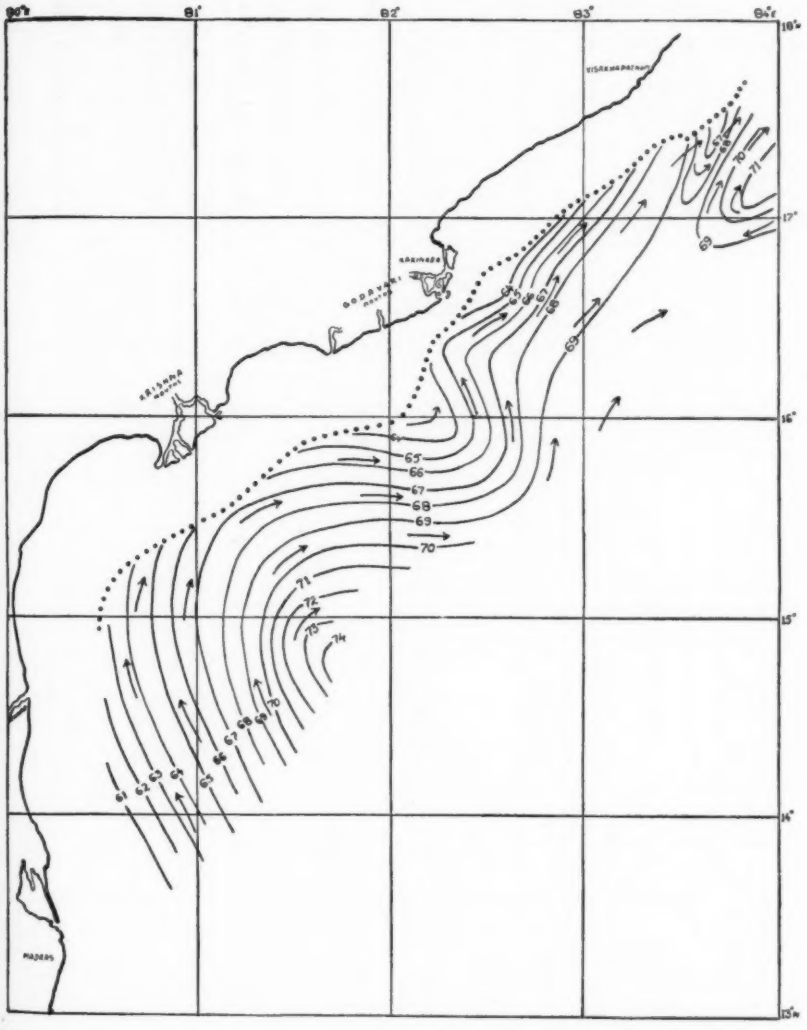


FIG. 20. Thermal field and the associated currents at 400 ft. depth level during the Hot Weather season along the east coast of India in the region between 13° N. to 18° N. and 80° E. to 84° E.

TABLE I

Analysis of bathythermograms at regions of current boundaries

Region No.	Season	Leve	Region of current boundary with description	Station		Nature of the bathythermograms
				No.	Position	
1	2	3	4	5	6	7
I	Post-Monsoon	Surface	Two stations in opposite directions off Madras	50	13° 17′ N. 18° 28·4′ E.	Temperature inversion upto 150 ft.
				51	13° 22′ N. 80° 33·5′ E.	Temperature inversion upto 200 ft.
				53	13° 32′ N. 80° 46·5′ E.	Temperature inversion upto 50 ft.
II	do.	100 ft.	About 60 miles off Visakhapatnam, a boundary between a clockwise gyrale and a north-westerly current at the gyrale	24 R	16° 45′ N. 83° 36′ E.	Temperature inversion between 50 and 100 ft. and double tracing between 50 and 150 ft. and steppy nature centered at 225 ft.
				24 U	16° 42′ N. 83° 33′ E.	Steppy nature between 150 and 250 ft.
III	do.	200 ft.	Off the Krishna, a boundary between easterly and westerly currents	24 Z	16° 37′ N. 83° 29′ E.	A number of steps between 50 and 150 ft.
				72	15° 48·5′ N. 81° 15·5′ E.	A double trace from 200 ft. downwards
				73	15° 47·7′ N. 81° 22′ E.	A sudden fall of temperature of about 5° F. from 200 to 220 ft. Steps below 200 ft. with a double trace
IV	do.	do.	Off Visakhapatnam, a boundary between a south-westerly and north-easterly current the latter being associated with an eddy	10	17° 24·3′ N. 83° 31·8′ E.	Boundary between inshore and the offshore currents, steps are shown
				12	17° 19·5′ N. 83° 35·7′ E.	Steps between 200 and 225 ft.
				18	17° 24′ N. 83° 31′ E.	Steps between 175 and 250 ft.
				23	17° 06′ N. 83° 48′ E.	Turbulent mixing and inversion upto 60 ft. and steps between 200 and 260 ft.

V	Post-Monsoon	200 ft.	Off Pennar near the coast a northerly to north-easterly current meets a south to south-westerly current	57	14° 06′ N. 80° 56′ E.	No steps
				58	14° 11′ N. 80° 47·3′E.	Steps between 250 and 400 ft.
				59	14° 16·5′ N. 80° 38·5′ E.	Sharp change in the vertical temperature gradient at 350 ft., steps centered at 150 ft. and isothermal layer at 200 ft.
VI	do.	400 ft.	Same as in V	60	14° 23·5′ N. 80° 22′ E.	Double tracing between 150 and 300 ft.
VII	North-east Monsoon	Surface	Eddy formation of Visakhapatnam	94	17° 30·2′ N. 83° 23·3′ E.	Same as above
				95	17° 28·8′ N. 83° 23·7′ E.	Temperature inversions at the surface
				129	17° 32·8′ N. 83° 26·7′ E.	Same as above
VIII	do.	40 ft.	Eddy formation off the Godavari	168	16° 57·9′ N. 82° 41·7′ E.	Small steps from surface to 75 ft.
IX	do.	100 ft.	Eddy formation off Visakhapatnam	135	17° 26·8′ N. 83° 31·1′ E.	Isothermal layer at this level
				136	17° 25·4′ N. 83° 32·3′ E.	
X	do.	Surface	Over the *Swatch of No Ground* convergence of currents into the canyan	115	21° 18·5′ N. 89° 27′ E.	Temperature inversion at the surface with steps between surface and 30 ft. Isothermal between 30 and 60 ft. Another inversion between 60 and 80 ft.
				119	20° 56′ N 89° 19′ E.	Isothermal between 80 and 90 ft. The rise in temperature is from 75° to 84° F.
				120	20° 55′ N. 89° 10′ E.	Indicating much turbulent condition. Temperature inversion with steps from surface to 300 ft. and double tracing in thermocline region
				124	20° 50′ N. 89° 05·5′ E.	
XI	do.	40 ft.	At the mouths of the Ganges	121	20° 53′ N. 88° 59′ E.	
				124	See above	See above

TABLE I (*Contd.*)

Region No.	Season	Leve	Region of curren tboundary with description	Station		Nature of the bathythermograms
				No.	Position	
1	2	3	4	5	6	7
XII	North-east Monsoon	100 ft.	A steep curvature in the currents over the *Swatch of No Ground*			Same as under region X
				293	16° 49·3′ N. 82° 20·8′ E.	Double tracing from surface to 100 ft.
XIII	Hot Weather	Surface	Eddy breaking off the Godavari	294	16° 49·3′ N. 82° 27·8′ E.	Small steps at surface
				296	16° 49·3′ N. 82° 34·2′ E.	Steps at 100 ft. Sharp fall of temperarure at surface. Steep at 200 ft.
				299	15° 49·3′ N. 82° 41′ E.	Steps in the upper 100 ft.
XIV	do.	do.	Eddy formation off Visakhapatnam	315	17° 29·5′ N. 83° 36·4′ E.	
				316	17° 27·5′ N. 83° 33·9′ E.	
XV	do.	40 ft.	Eddy formation off the Godavari	Ref. XIII		Same as under region XIII
XVI	do.	100 ft.	Eddy formation off Visakhapatnam	207	17° 25·5′ N. 83° 34·4′ E.	Steps from surface to 200 ft. and double tracing down to 400 ft.
				208	17° 23·9′ N. 83° 36′ E.	Steps in the mixed layer
XVII	do.	200 ft.	Off Visakhapatnam, southerly to south-westerly current meets with a northerly current	212	17° 20·8′ N. 83° 52·1′ E.	Steps from surface to 200 ft.
				211	17° 19·7′ N. 83° 40·5′ E.	Steps at 300 ft.

coast. Hence the extent and the duration of the presence of this water on the continental shelf off Visakhapatnam is greater than in the south (*i.e.*, say, off the Godavari). An examination of the isotherms at 100 and 200 feet levels, in a region between north of the Godavari and south of Visakhapatnam, reveals the intensity of the current at these levels. But in the surface waters, even though the strength of the current is not weak, much turbulence appears. Further south in this region the isotherms are widely spaced except in the 400 feet level chart (Fig. 20) where the isotherms are nearly uniform in the entire chart.

Thus the current is weak and north-easterly in the south and intense and turbulent in the north off the Godavari upto 200 feet level while at 400 feet the current is nearly uniform in intensity and is northerly upto the Krishna and becomes easterly upto the Godavari and north of the Godavari it turns north-westerly and finally it becomes a north-easterly current.

Current Boundaries.—A current boundary may be defined as a region where two currents from opposite or nearly opposite directions meet. In such a case, different watermasses with different conservative properties such as salinity or temperature come in juxtaposition. If the vertical distribution of the temperature or salinity is studied in such regions, one will find, often, steps in the respective profiles. This characteristic feature can be traced in bathythermograms also. In Table I, the regions of current boundaries that are noticed at each level have been indicated. Where observations are available in such regions, the bathythermograms have been analysed. The analysis of the bathythermograms has been entered in the last column of Table I. It will be found from this analysis that the bathythermograms either show steps in a layer whose mean depth is round about the depth of the level under consideration, or the bathythermogram shows two different lines separated by about one degree difference in temperature. This is due to the fact that each tracing is made as the instrument is lowered into and heaved up. In the short interval of the downward and upward travel of the instrument the different watermasses give their characteristic temperature to the thermal element of the instrument, thus resulting in two curves side by side. This analysis not only gives a method of exactly locating the current boundaries but also serves as a verification of the validity of the method employed to draw conclusions regarding the possible directions of currents from the thermal field, provided no consideration is taken of the bathythermograms which are showing the expected characteristic. While drawing the isotherms, actually the mean temperatures are taken from such bathythermograms.

From Table I it is seen that no boundary is exhibited by the bathythermograms in the region I. This is so because the two rotating systems are seen as mainly of thermal origin. It has been already shown in the present investigation (by salinity consideration) that such rotating current system does not exist to any appreciable extent. However at all other regions considered, current boundaries exhibited by the bathythermograms provided a check over the validity of the currents entered. However, further investigation, either by direct measurements or dynamical computations, is highly desirable.

4. SEASONAL VARIATION

The temperature of the sea is effected by the processes of heat transfer, *viz.*, the advective processes and the energy transfer. In the surface layers, where the effect of solar radiation is very intense, the processes of energy transfer are of utmost importance. The time and space means of the sea surface temperature and air temperature together with the difference for every month have been worked out by Ramasastry (unpublished). His values have been reproduced in Table II. From this table it is seen that the sea

TABLE II

Comparison of sea surface and air temperatures

Month	Temperatures ° F.		Difference
	Sea surface	Air	Columns 2 – 3
1	2	3	4
October ..	83·1	84·3	−1·2
November ..	80·7	80·8	−0·1
December ..	77·5	80·9	−3·4
February ..	80·7	83·2	−2·5
March ..	81·6	82·9	−1·3
April ..	83·8	85·5	−1·7

surface is always colder than the air in all the three seasons, the greatest difference being 3·4° F. in December. This means that heat is always transferred from the atmosphere to the sea, the maximum transfer being in winter.

But the thermal field shows some peculiarity which reflects the various processes that are responsible for the changes in temperature of the sea. This is well brought out by a level-to-level comparison of the temperature fields in the three seasons under discussion. For this purpose in Table III, the maximum and minimum values of temperature for each level in different seasons have been indicated (from the charts prepared, the temperatures are given in whole ° F.). Also the range of temperature and the place of occurrence of maximum and minimum temperature zones are given.

As a detailed discussion of the thermal field and the associated currents has been presented in the earlier pages, the seasonal characteristics have been briefly summarised in Table III, on which a discussion is presented here.

Discussion of Table III.—During the Post-Monsoon season sea surface temperature begins to fall gradually reaching a minimum early in the North-east Monsoon season. The difference between the sea surface and air temperatures becomes reduced towards the end of this season and sinking gains in intensity during the middle of the season. Due to this, the topmost layers are characterised by nearly uniform temperature along the coast, while off-shore the temperatures are much less as is indicated by column 5 in Table III. The maximum range of temperature occurs at 200 feet level. This is because the thermocline develops almost round this depth near the coast as a consequence of sinking, while it is much shallower well off into the Bay, where sinking is not much prominent.

During the North-east Monsoon season, the sea surface temperature shows a minimum as a result of surface cooling by the reduction in the intensity of the solar radiation. The subsurface layers do not cool down and hence a subsurface zone of maximum temperature, as was presented by LaFond and Borreswara Rao (in print), has formed, not only in the east coast of India (to which region they have confined their studies) but also at the head of the Bay. From column 9 in Table III, it is seen that at all levels highest temperatures occur towards the coast at the estuaries of the Ganges and a low temperature just on the submarine canyon. On the boundary of the canyon the presence of vertical instability in the top layers of the sea has been found by Ramasastry (unpublished). Due to this instability uniform temperatures in the top layers must result as a consequence of vertical mixing. But on the contrary the temperature increases with depth indicating the extent of surface cooling due to the advent of winter. The maximum range of temperature is found only in the topmost layer, whereas in the Godavari-Visakhapatnam region it is found at 200 feet level as in the previous season.

TABLE III

Seasonal Characteristics of the Thermal Field

1	Post-Monsoon Season				North-east Monsoon Season				Hot Weather Season			
	Temperature °F.			Region occurring max. or min.	Temperature °F.			Region occurring max. or min.	Temperature °F.			Region occurring max. or min.
	Max. 2	Min. 3	Range 4	5	Max. 6	Min. 7	Range	9	Max. 10	Min. 11	Range 12	13
Surface	86·0	79·0	7·0	Upto about 20 miles off Visakhapatnam	82·0	79·0	3·0	Off the Godavari	84·0	79·0	5·0	In the coastal region south of Visakhapatnam
					79·0	74·0	5·0	Near the mouths of Ganges				
40 ft.	85·0	80·0	5·0	Upto 40 miles off Visakhapatnam and at the estuary of Godavari (83°)	81·0	79·0	6·0	Centered at about 20 miles off Visakhapatnam and off Godavari	84·0	79·0	5·0	Centered at 17° 35′ N. and 83° 20′ E. and about 30 miles off Kakinada
					80·0	74·0	6·0	Lowest temp. here and surrounded by higher temperatures				
100 ft.	84·0	80·0	4·0	In a narrow zone south of Visakhapatnam and north of Kakinada	80·0	78·0	2·0	Off Visakhapatnam	82·0	74·0	8·0	Low temperatures over the continental shelf between Visakhapatnam and the Godavari
					83·0	80·0	3·0	Head of the Bay				
200 ft.	84·0	71·0	12·0	Off the Krishna along narrow zone at 200 ft. line	80·0	73·0	7·0	Low temp. off Kakinada and high temp. off Visakhapatnam	81·0	69·0	12·0	Very low temps. off Kakinada along the 200 ft. line and upto Visakhapatnam
					84·0	80·0	4·0	High temps. towards the coast at the head of the Bay				High temps. in the open Bay off Madras
400 ft.	67·0	59·0	8·0	Max. temp. south off the Godavari and along the 400 ft. line	71·0	69·0	2·0	Higher temp. near the 400 ft. line	64·0	74·0	10·0	High temps. in offshore waters
					78·0	75·0	3·0	Higher temps. away from the coast				

N.B..—The 2nd row in columns 6–9 refers to the conditions at the *Swatch of No Ground.*

Owing to the increased intensity in the solar radiation towards the end of winter and with the change in the direction of the surface winds, mixing in the top layers is completed by the end of February and the subsurface zone of maximum temperature is completely eliminated. Upwelling starts and gains in intensity during this period, so much so the depth of thermocline will be shallower near the coast than offshore. The conditions will be just the reverse to those in the Post-Monsoon season. This is made clear by comparing columns 5 and 13 in Table III. During this season also, the maximum range of temperature occurs at 200 feet level.

References

1. LaFond, E. C. .. "On upwelling and sinking off the east coast of India," *Andhra University Memoirs in Oceanography*, Andhra University, Series No. 49, 1954.

2. ——— and Borreswara Rao, C. "Vertical temperature structure of the upper layers of the sea off the east of coast of India," *Defence Science Journal* (in print).

3. Ramasastry, A. A. .. "Vertical temperature structure of the sea along the south coast of India," *Ibid.*, October, 1954.

4. ——— .. "Vertical temperature structure in the north-western Bay of Bengal and over the *Swatch of No Ground.*" (To be published.)

5. ——— .. "Vertical temperature gradients in the Bay of Bengal and the Arabian Sea." (To be published.)

6. ——— .. "Diurnal variation of the temperature structure of the sea." (To be published.)

7. Sewell, R. B. S. .. "Temperature and salinity of surface waters in the Bay of Bengal and Andaman Sea with reference to Laccadive Sea," *Memoirs of the Asiatic Society of Bengal*, 1929, 9 (5), 207–351.

8. Sverdrup, H. U., Johnson, M. W. and Fleming, R. H. *Oceans, Their Physics, Chemistry and General Biology*, Prentice-Hall, Inc., New York, 1946.

| | | |

HYPHOMYCETES—IV*

By C. V. Subramanian, F.A.Sc.
(*University Botany Laboratory, Madras-5*)

Received August 14, 1957

50. **Arthrobotryum nilgirense** sp. nov.

This fungus was found growing saprophytically on dead bamboo at the Sim's Park, Coonoor, during a visit in November 1956. It forms dark conspicuous colonies consisting of scattered synnemata. The synnemata are usually simple, erect, straight, consisting of a stalk and a capitate, broadened, fertile head. The stalk is somewhat cylindrical, rigid, tall, with a broadened base, dark opaque, composed of numerous simple, septate, brown, thin hyphæ closely aggregated together, 560–1,302 μ tall inclusive of the head, 56–196 μ wide at the base, 28–63 μ wide above and 28–70 μ wide immediately below the head. The heads are usually elongate-clavate, opaque black, 168–406 μ tall and 70–224 μ wide where they are widest. The heads are composed of conidiophores and conidia borne on them. The conidiophores are the free ends of the hyphæ of the synnema and they are of variable length, brown in colour, septate, somewhat clavate and dark brown towards the tip, bearing conidia singly and acrogenously. Each hypha of the synnema in most cases terminates in one conidiophore; in other cases it may, in addition, give out one or more conidiophores arising usually from immediately below the penultimate septum in the main hypha. The conidia are obclavate to fusiform or irregular in shape, straight, curved or bent, usually 4–6-septate when mature, with roughened outer wall, with all cells except the apical one usually dark brown in colour, the apical cell being paler and subhyaline, with a somewhat flat basal scar indicating the point of attachment to the conidiophore, broadest towards the base or middle and tapering above sometimes into an elongate-conical apical cell. The conidia are 26·6–50·8 μ long, 6·3–8·4 μ wide (where widest), each with a basal scar 2·1 μ wide; the basal cell is 4·9–5·6 μ wide; the apical cell is 4·9–14·0×2·8–5·6 μ.

The aggregation of dark brown hyphæ to form synnemata which bear distinct heads of conidia and the production of phæophragmospores singly and acrogenously on the conidiophores indicate that the fungus may be placed in the genus *Arthrobotryum*. It has features quite distinct from

* Hyphomycetes I, II and III were published in *J. Indian bot. Soc.*, **35**, 53–91, 446–94 and **36**, 61–67, respectively.

species of this genus so far known and is, therefore, classified here as a new species:

Arthrobotryum nilgirense Subramanian sp. nov.

Coloniæ fuscæ, constantes e synnematibus dispersis. Synnemata ut plurimum simplicia, erecta, recta, constantia e stipite atque capitulo latiore fertili. Stipes aliquantum cylindricus, basi latiori, fusce haud pellucidus,

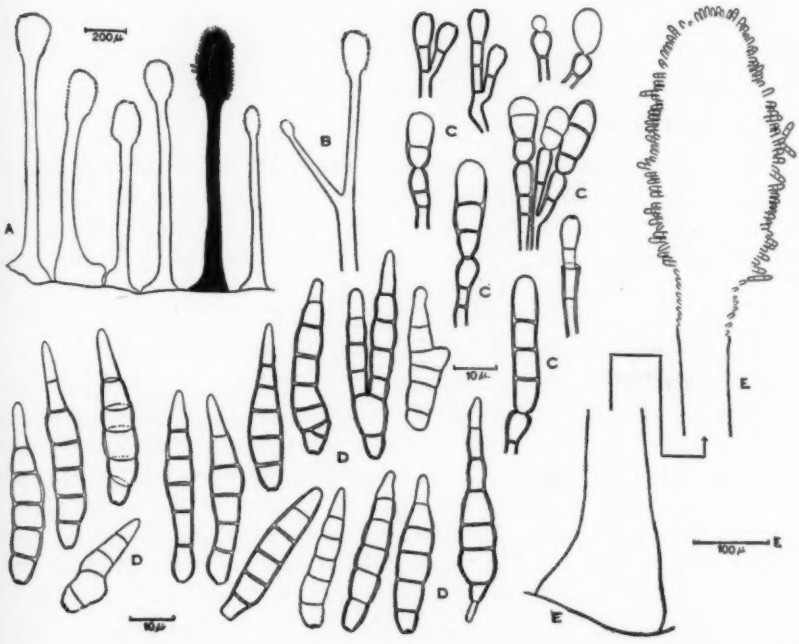


FIG. 1. *Arthrobotryum nilgirense* from type specimen, Herb. M.U.B.L. 1738. A, synnemata; B, portion of a branched synnema; C, conidiophores and stages in the development of conidia; D, mature conidia; E, enlarged drawing of a synnema.

constans hyphis plurimis simplicibus, septatis, tenuibus, brunneis arcte aggregatis, capitulo incluso 560–1,302 μ altus, 56–196 μ latus ad basim, 28–63 μ latus supra, 28–70 μ latus sub ipso capitulo. Capitulum ut plurimum elongato-clavatum, nigrum, 168–406 μ altum, 70–224 μ latum latitudine maxima, constans e conidiophoris conidia ferentibus. Conidiophori (qui sunt apices liberi hypharum synnematis) longitudinis variæ, brunnei, septati, aliquantum clavati atque fusce brunnei ad apicem. Conidia singulariter atque acrogene producta e conidiophoris, obclavato-fusiformis vel irregulalaria, recta, flexa vel curvata, ut plurimum 4-6-septata ad maturitatem,

parietibus externis asperis, cellulis omnibus una apicali excepta fusce brunneis, apicali vero pallidiori atque subhyalina, cicatrice basali aliquantum complanata monstrante punctum unionis cum conidiophoro, latissima ad basim vel ad medium atque fastigata supra haud raro in cellulam apicalem elongato-conicam; conidia 26·6–50·8 μ longa, 6·3–8·4 μ lata ad partem latiorem, singula ornata cicatrice basali 2·1 μ lata; cellula basalis conidii 4·9–5·6 μ lata; cellula vero apicalis conidii 4·9–14·0×2·8–5·6 μ.

Typus lectus in Bambusa emortua, in loco Sim's Park, ad Coonoor, in regione Nilgiris, in Statu Madras, die 22 novembris anni 1956 a C. V. S. et positus in Herb. M.U.B.L. sub numero 1738.

51. **Ceratosporella deviata** sp. nov.

The fungus forms conspicuous, dark, somewhat velutinous colonies on the substratum (dead leaf base of *Raphilostyles sapida*). The conidiophores are simple, erect, straight, dark brown, septate (up to 3 septa), cylindrical, thick-walled, smooth, and arising from a highly lobed, dark brown basal cell. The basal cells are of variable length and are 10·5–12·6 μ wide. The conidiophores are up to 5·6 μ wide at the base, 4·2–4·9 μ wide in the middle, abruptly narrowed and about 2·8 μ wide at the tip, and 126–210 μ long. The conidia are produced singly and acrogenously at the tips of the conidiophores. Apical proliferation of the conidiophores through scars of fallen conidia is common. The conidia are brown, mostly three-armed, sometimes two- or four-armed, the arms arising from a somewhat obpyriform basal cell with a short or long basal prolongation, and are attached to the conidiophores directly by their basal cells. The basal cell of each conidium is thick-walled and brown. The conidial arms are of variable length, darker, thicker walled and wider towards the base, septate (up to 4-septate), and often constricted at septa. The apical (central) arm is 22·4–37·8 (—61·6) μ long and 5·6–7·0 (—7·7) μ wide; the lateral arms are 16·8–43·4 μ long and 4·9–7·0 μ wide.

The details of the development of the conidia appear to be as follows. A swelling appears at the tip of the conidiophore. This swelling elongates and is cut off from the conidiophore by a septum. The swelling becomes narrow-obclavate in shape and a lateral swelling then appears about the middle or just below the middle part of the swelling. One septum appears in the main swelling immediately above the region of the origin of the lateral swelling, thus cutting off a basal cell for the conidium. Elongation and further septation of the primary swelling results in the apical arm of the conidium. The lateral swelling elongates and in the meanwhile another lateral swelling appears on the basal cell of the conidium. These lateral

swellings elongate, are cut off by septa from the basal cell of the conidium and become septate, forming the lateral arms of the mature conidium. The cells of the apical and the lateral arms are wider towards their base than

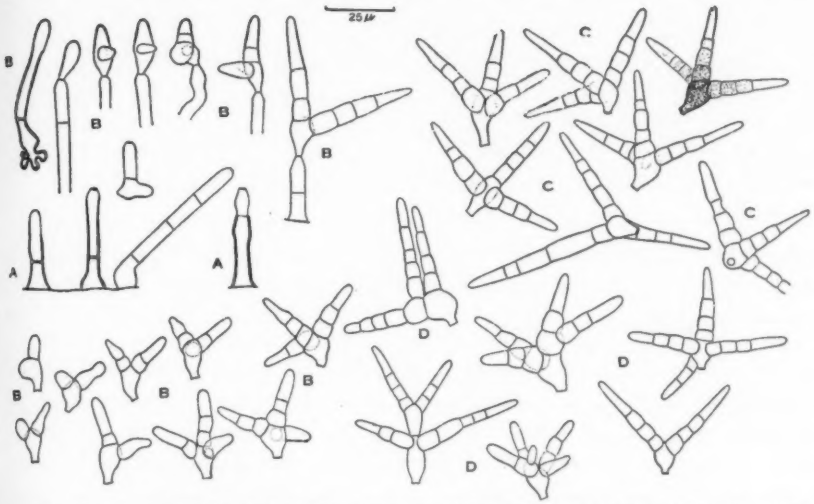


FIG. 2. *Ceratosporella deviata* from type specimen, Herb. M.U.B.L. 1778. A, conidiophores; B, stages in the development of conidia; C, normal mature conidia; D, abnormal mature conidia.

above and the apical cells of these arms are long-obconical and have smoothly rounded ends.

The fungus just described has a close resemblance to *Triposporium elegans* Corda; but a critical analysis of its features indicates that it is probably identical with the *Ceratosporella* sp. described by Hughes (1951). Hughes described and figured *Ceratosporella* sp. (? nov.) from a collection labelled "*Triposporium elegans* Ca. with *Microthyrium*. 2,437 on *Quercus* (leaves, probably from California) Harkness (scripsit Cooke)" ex Herb. R.B.G., Kew. The presence of a distinct stalk cell in the conidium and repeated apical proliferation of conidiophores are features found in *Ceratosporella bicornis* (Morgan) Hoehnel, the type species of the genus *Ceratosporella* Hoehnel (*see* Hughes, 1951) and I follow Hughes in classifying this fungus in *Ceratosporella*. My fungus is quite distinct from *C. bicornis*, but is probably the same as *Ceratosporella* sp. described by Hughes. It is classified here as a new species:

Ceratosporella deviata Subramanian sp. nov.

Coloniæ fuscæ, velutinæ. Conidiophori simplices, erecti, recti, fusce brunnei, septati (septis usque 3), cylindrici, crassis parietibus præditi, leves,

surgentes e cellula alte lobata, fusce brunnea, basalique, usque ad 5·6 μ lati ad basim, 4·2–4·9 μ ad medium, 2·8 μ ad apicem, 126–210 μ longi; cellula basalis 10·5–12·6 μ lata, longitudinis variæ. Conidia producta singulariter atque acrogene, brunnea, ut plurimum ter brachiata, nonnumquam bis vel quater brachiata, brachiis surgentibus e cellula basali aliquantum obpyriformi prædita brevi longave prolongatione basali; conidii cellula basalis crassis parietibus prædita, brunnea; brachia longitudinis variæ, obscuriora, parietibus crassioribus et latioribus ad basim prædita, septata (usque quater) atque sæpe constricta ad septa; brachium apicale (centrale) 22·4–37·8 (—61·6)×5·6–7·0 (—7·7) μ; brachia lateralia 16·8–43·4×4·9–7·0 μ.

Typus lectus in foliis emortuis *Raphilostyles sapidæ* in loco Government Garden, Ootacamund, in regione Nilgiris, in Statu Madras, die 24 novembris anni 1956 a C. V. S. et positus in Herb. M.U.B.L. sub numero 1778.

52. **Periconia nilagirica** sp. nov.

The fungus forms scattered groups of conidiophores with conidia, on the substratum. Each conidiophore consists of a stipe bearing a head of conidia at its tip. The stipe is erect, straight, bent or curved, smooth, thick-walled, brown in colour, darker towards the base, slightly paler towards

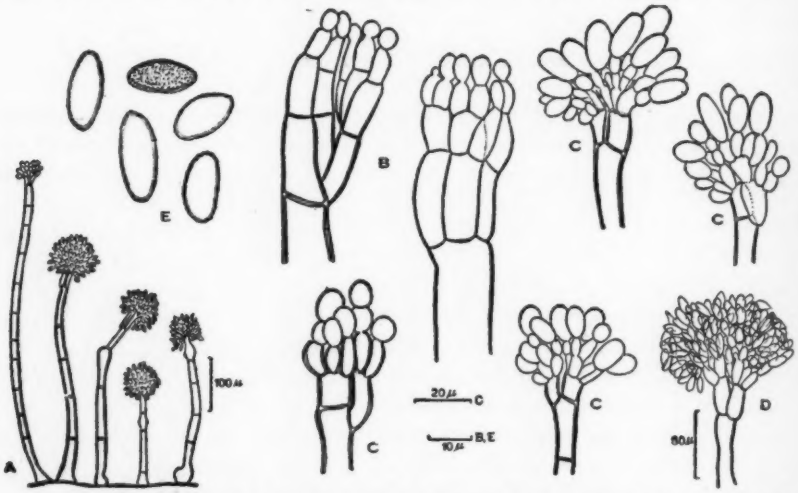


FIG. 3. *Periconia nilagirica* from type specimen, Herb. M.U.B.L. 1621. A, conidiophores and heads of conidia; B, C, development of conidia; D, head of conidia; E, mature conidia.

the apex, broad or swollen and 21–25 μ wide at the base, cylindrical and 9·8–14·0 μ wide immediately above the swollen base and further up, 168–560 μ long, septate, simple but often producing short lateral branches singly or in

twos or threes below the apex beneath transverse septa. The head is globose or irregular in shape; it is 36·0–111·6 μ high and 45·8–126·0 μ wide. The apical branches of the stalk are cylindrical, tapering towards the distal ends, at first adpressed, later spreading, brown at the base, paler above, usually producing secondary and tertiary branches; the primary branches are 16·8–21·0 × 8·4–9·1 μ; the secondary branches are up to 9·8×5·6 μ; the tertiary branches up to 8·4×4·9 μ. The conidia are always borne in simple or branched acropetal chains at the tips of the ultimate branches, maturing from the apex downwards. The mature conidia are dark brown, fusiform with rounded ends, markedly echinulate, thick-walled, one-celled and 14–21 ×7·0–9·8 μ.

It is obvious that the fungus is a *Periconia* with fusiform spores. It comes close to *P. sacchari* Johnston (*see* Saccardo, 1931, p. 768). Through the courtesy of Dr. J. A. Stevenson of the National Fungus Collections, U.S.D.A., I have been able to examine type material of *P. sacchari*, but it appears to be somewhat different from my fungus. *P. sacchari* has conidiophores up to 840 μ long or more, and the conidia are mostly 22×11 μ, whereas in my fungus the conidiophores are seldom longer than 560 μ and the majority of conidia are 16·8×8·4 μ. I am, therefore, classifying my fungus as a new species of *Periconia*.

Periconia nilagirica Subramanian sp. nov.

Conidiophori dispersi in substratum atque constantes e stipite atque apice capitato fertili. Stipes erectus, rectus, flexus vel curvatus, levis, crassis parietibus præditus, brunneus, obscurior ad basim, tenuiter pallidior ad apicem, latus vel tumescens atque 21–25 μ latus ad basim, cylindricus atque 9·8–14·0 μ latus supra ipsam basim tumescentem atque superius, 168–560 μ longus, septatus, simplex vel sæpe ramis lateralibus brevibus nonnullies ornatus ad apicem infra septa; rami cylindrici fastigati ad apicem, primo adpressi, tum patentes, brunnei, ut plurimum producentes ramos secundarios et tertiarios; ramuli primarii 16·8–21·0×8·4–9·1 μ; secundarii vero usque ad 9·8×5·6 μ; tertiarii ad 8.4×4·9 μ. Capitula conidiorum forma irregulari, 36·0–111·6×45·8–126·0 μ. Conidia semper apicibus ramulorum ultimorum insidentia acropetale catenulata, maturitatem attingentia ex apice deorsum; matura conidia fusce brunnea, fusiformia, apicibus rotundatis, distincte echinulata, crassis parietibus prædita, semel-cellulata, 14–21×7·0–9·8 μ.

Typus lectus in culmis emortuis graminum, in loco Government Garden, Ootacamund, in regione Nilgiris, in Statu Madras, die 24 mensis septembris anni 1955 a C. V. S. et positus in Herb. M.U.B.L. sub numero 1621.

53. **Stachybotrys nilagirica** sp. nov.

This fungus was collected on dead twigs of *Althæa rosea* at the Sim's Park, Coonoor, during a visit in November 1956. It produces scattered conidiophores on the substratum. The conidiophores (phialophores) are erect, straight, hyaline, subcylindrical, being slightly narrower above than below, 1–4-septate (septa 18·0–61·2 μ apart), swollen and 9·8–12·6 μ wide at the base, 8·4–9·8 μ wide above, 112–224 μ long, thick-walled below, thin-walled above, smooth, slightly swollen and 6·3–7·0 μ wide at the tip, and terminating in a cluster of about 6–7 phialides. The apical cell of the phialophore is 50·4–61·2 μ long. The phialides are short-clavate, subhyaline when young, pale olivaceous green when mature, smooth, 12·6–15·4 μ long and 7·0–8·4 μ wide. The conidia are borne singly and acrogenously at the apices of the phialides and are one-celled, globose, markedly tuberculate, dark greenish black when mature, 15·4–28·0 (mostly 21·0–25·2) μ in diameter.

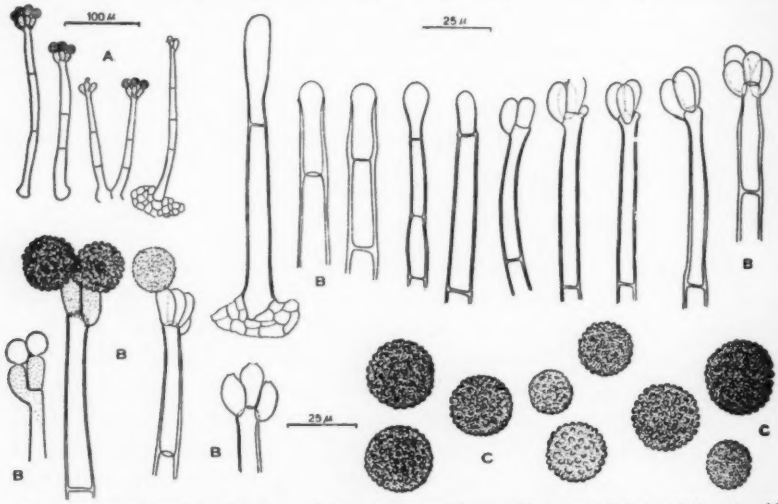


FIG. 4. *Stachybotrys nilagirica* from type specimen, Herb. M.U.B.L. 1813. A, phialophores; B, stages in the development of phialides and phialospores; C, mature phialospores.

The growth of the phialophore is terminated by the production of a terminal phialide usually 11·2–12·6 μ long and 7·0–8·4 μ wide. Later, 1–6 phialides develop from immediately below the apical phialide just below the septum, thus forming an apical cluster.

Comparison of the features of my fungus with those of species of *Stachybotrys* indicates that the former is distinct from *Stachybotrys* spp. so far known. My fungus is, therefore, treated here as a new species.

Stachybotrys nilagirica Subramanian sp. nov.

Conidiophori erecti, recti, hyalini, subcylindrici, simplices, 1–4-septati (septis inter se 18·0–61·2 μ distantibus) tumescentes atque 9·8–12·6 μ lati ad basim, 8·4–9·8 μ lati supra, 112–224 μ longi, crassis parietibus infra, tenuibus vero supra ornati, leves, aliquantum tumescentes et 6·3–7·0 μ lati ad apicem, desinentes in fasciculum 6–7 phialidum. Phialides brevi-clavatæ, juniores quidem subhyalinæ, vetustiores vero pallide olivaceo-virides, leves, 12·6–15·4×7·0–8·4 μ. Conidia singulariter atque acrogene insidentia apicibus phialidum, semel cellulata, globosa, distincte tuberculata, fusce viridi-nigra ad maturitatem, 15·4–28·0 (ut plurimum 21·0–25·2) μ diam.

Typus lectus in ramulis emortuis *Althææ roseæ* Cav. in loco Sim's Park, ad Coonoor, in regione Nilgiris, in Statu Madras, die 22 novembris anni 1956, a C. V. S. et positus in Herb. M.U.B.L. sub numero 1813.

54. **Drumopama girisa** gen. et. sp. nov.

The fungus forms scattered synnemata on both surfaces of decaying and dead leaves of Gramineæ, sometimes in groups. The synnemata are erect, dark, consisting of a stalk broadened and 25·2–36·0 μ wide below the base, 14·4–18·0 μ wide at the base, 14·4–21·6 μ wide in the middle and are 434–1,332 μ long. The hyphæ of the synnema are thin, simple, septate, pale brown, and become free above at varying lengths from the base and these free hyphæ are the conidiophores bearing conidia. The stalk of the synnema is 266–364 μ tall, cylindrical and dark in colour. The free conidiophores vary considerably in length, being 56–882 μ long; they are simple, straight, curved, bent or flexuose, dark brown, paler to subhyaline towards the tip, cylindrical and somewhat of uniform thickness, 3·5–4·2 μ wide at the base, 2·8–4·2 μ wide towards the tip, septate towards the base (septa 168–280 μ apart) and markedly geniculate. The conidia are produced singly and acrogenously; they are ovoid to subglobose, with a basal papilla indicating the point of attachment to the conidiophore, hyaline, smooth, 8·4–12·6 μ long and 7·0–9·8 μ wide. The conidiophore elongates from a point just below the scar of the first acrogenous conidium and after growing for a length produces another conidium acrogenously. Successive production of conidia in this way gives the fertile part of the conidiophore the geniculate appearance.

The fungus is obviously a member of the Moniliales-Phæostilbeæ. It has some resemblance to the genus *Melanographium* Sacc. (= *Sporostachys* Sacc.) (*see* Saccardo, 1931, p. 936). Through the courtesy of Dr. J. A. Stevenson of the National Fungus Collections, U.S.D.A., I have examined the type specimen of *Sporostachys maxima* Sacc. and I find that it produces

synnemata composed of closely aggregated parallel brownish hyphæ which become free above and produce one-celled, brown, convex, lenticular conidia (similar to those of genera such as *Pseudocamptoum*, *Papularia*, *Arthrinium*,

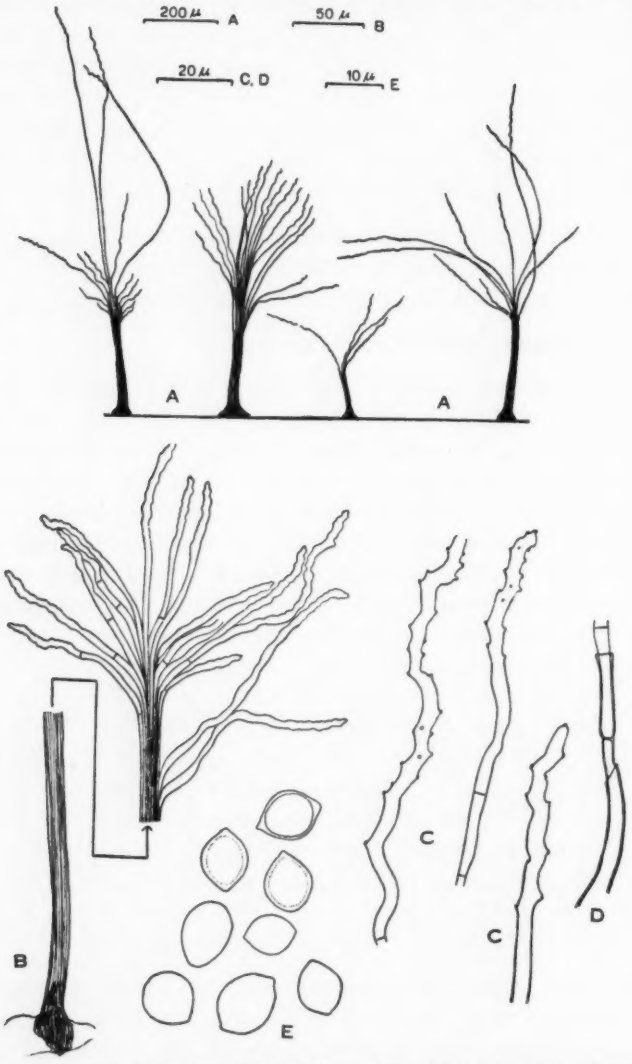


FIG. 5. *Drumopama girisa* from type specimen, Herb. M.U.B.L. 1753. A, synnemata; B, enlarged drawing of a synnema; C, fertile parts of conidiophores; D, proliferation of conidiophore; E, mature conidia.

etc.) acrogenously. In my fungus, the conidia, although one-celled, are hyaline and are not lenticular. I consider the lenticular nature of conidia of fungi to be of generic significance. For this reason, I do not place my fungus in *Melanographium* (= *Sporostachys*). The genus *Tharoopama* recently described by me (Subramanian, 1956) belongs to the Phæostilbeæ-Amerosporæ and produces hyaline one-celled conidia acrogenously on conidiophores which are the branched free ends of the hyphæ of the synnemata. The unbranched conidiophores of my fungus distinguish it from the type species of the genus *Tharoopama*, *T. trina* Subram., whose conidiophores are profusely and intricately branched. I know of no genus of the Phæostilbeæ in which the present fugnus can be suitably classified. I am, therefore, proposing a new genus for it. The generic and specific names are both derived from Sanskrit: the generic name *Drumopama* from द्रुम (*druma*) = tree, and उपम (*upama*) = like, from the tree-like appearance of the synnemata: the specific epithet *girisa* from गिरिश (*girisa*) = dwelling in the mountains, indicative of the occurrence of the fungus at an altitude over 7,000 feet above sea-level.

Drumopama Subramanian gen. nov.

Fungus imperfectus, Moniliales, Phæostilbeæ, Amerosporæ.

Synnemata simple, dark, erect, composed of simple, septate, brown hyphæ becoming free above. Conidiophores (free ends of hyphæ of synnema) simple, brown, geniculate. Conidia one-celled, hyaline, produced acrogenously and singly.

Pertinet ad Fungos Imperfectos, ad Moniliales, Phæostilbeas, Amerosporas. Synnemata simplicia, fusca, erecta, constantia e hyphis simplicibus, septatis, brunneis, quæ supra liberæ evadunt. Conidiophori (apices liberi hypharum synnematis) simplices, brunnei, geniculati. Conidia semel cellulata, hyalina, singulariter et acrogene producta. Species typica sequens.

Drumopama girisa Subramanian sp. nov.

Synnemata erecta, fusca, 434–1,332 μ alta, constantia e stipite atque parte fertili superiore quæ constat e conidiophoris (apicibus liberis hypharum synnematis) conidia ferentibus. Stipes cylindricus, latior ad basim (25·2–36·0 μ latus), 14·4–18·0 μ latus supra ipsam basim, 14·4–21·6 μ latus supra, atque 266–364 μ altus. Hyphæ synnematis tenues, septatæ, simplices, pallide brunneæ, evadentes liberæ supra ad efformandos conidiophoros. Conidiophori longitudinis variæ, 56–882 μ longi, simplices, recti, flexi vel curvati, fusce brunnei, pallidi ad hyalinos ad apicem, cylindrici atque plus minusve

uniformes crassitudine, 3·5–4·2 μ lati ad basim, 2·8–4·2 μ lati ad apicem, septati ad basim (septis 168–280 μ inter se distantibus), distincte geniculati. Conidia producta acrogene atque singulariter, ovoidea ad subglobosa, papilla basali monstrante punctum unionis cum conidiophoris, hyalina, levia, 8·4–12·6 μ longa, 7·0–9·8 μ lata.

Typus lectus in foliis emortuis graminum in loco Government Garden, ad Ootacamund, in regione Nilgiris, in Statu Madras, die 19 novembris anni 1956 a C. V. S. et positus in Herb. M.U.B.L. sub numero 1753.

55. *Harpographium fasciculatum* Sacc., 1880, *Michelia*, **2**, 33; *Fungi ital.*, t. 13; 1884, *Sylloge Fungorum*, **4**, 619.

The fungus forms conspicuous synnemata on dead stems. The synnemata are gregarious, arising from a common stromatic base, dark brown, erect, straight or bent, somewhat cylindrical to conical, up to 560 μ tall, 42–98 μ wide at the base, 28–84 μ wide in the middle, up to 42 μ wide towards the tip, fertile all along the length except for a short distance from the base

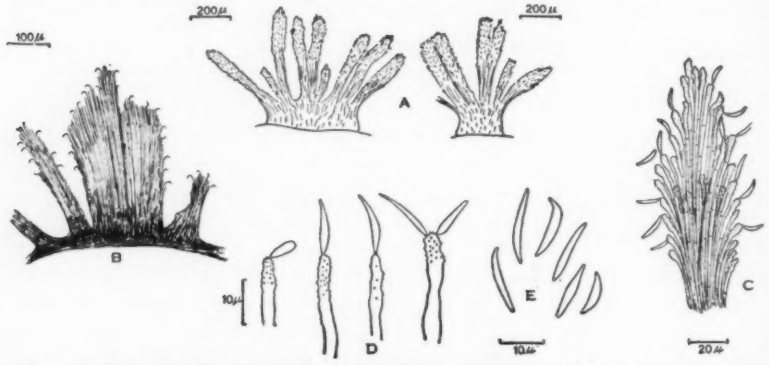


FIG. 6. *Harpographium fasciculatum* from Herb. M.U.B.L. 1784. A, B, groups of synnemata; C, apical part of a synnema; D, fertile apices of conidiophores bearing conidia; E, mature conidia.

and composed of pale to dark brown simple hyphæ. The apical parts of these hyphæ are free and do not lie closely adpressed or parallel to the synnematal strands. They are sometimes somewhat verrucose above, septate, 1·4–2·1 μ wide, 2·8–4·2 μ wide towards the tip which has distinct conidial scars crowded together or with fewer ones and then presenting a distinct geniculate appearance. The scars indicate the points of attachment of fallen condia. The conidia are produced singly and acropleurogenously, and are one-celled, fusiform to falcate, hyaline, 7·0–15·4 μ long and 1·4–2·1 μ wide.

Habit.—On dead stems, Sim's Park, Coonoor, Nilgiris, Madras State, 22 November 1956, coll. C. V. S., Herb. M.U.B.L. 1737 and 1784.

This is apparently the first record of this fungus from India.

Summary

The following five new species of Hyphomycetes, collected from the Nilgiris, Madras State, are described in this paper: *Arthrobotryum nilgirense* on dead bamboo, *Ceratosporella deviata* on dead leaf base of *Raphilostyles sapida*, *Periconia nilagirica* on dead culms of grass, *Stachybotrys nilagirica* on dead twigs of *Althæa rosea*, and *Drumopama girisa* on dead leaves of Gramineæ. Of these, *D. girisa* is proposed as a gen. et sp. nov. and is placed in the Moniliales–Phæostilbeæ–Amerosporæ. *Harpographium fasciculatum* Sacc. is recorded from India for the first time.

Acknowledgements

I am deeply indebted to the Rev.-Fr. Prof. H. Santapau of the St. Xavier's College, Bombay, for kindly providing Latin translations for the generic and specific diagnoses, and to Dr. J. A. Stevenson of the National Fungus Collections, U.S.D.A., for kindly having sent me for study specimens in his keeping.

References

Hughes, S. J. .. "Studies on micro-fungi. XII. *Triposporium*, *Tripospermum*, *Ceratosporella* and *Tetraposporium* (gen. nov.)," *Mycol. Pap.*, 1951, **46**, 35 pp.

Saccardo, P. A. .. *Sylloge Fungorum*, 1931, **25**, 1093 pp., litho-printed by Edwards Brothers, Ann Arbor, Michigan.

Subramanian, C. V. .. "Hyphomycetes—I," *J. Indian bot. Soc.*, 1956, **35**, 53–91.

TÆNIOMA J. AG. FROM INDIA

By T. V. Desikachary, F.A.Sc. and M. S. Balakrishnan
(*University Botany Laboratory, Madras-5*)

Received August 14, 1957

In 1848, J. Agardh described an alga from the Pacific coast of Mexico as *Polysiphonia perpusilla*. Later, in 1863, he created the genus *Tænioma* based on this species. A second species, *T. macrourum*, was described by Thuret (in Bornet and Thuret, 1876) from Tangier, Morocco. Farlow (1877) described a third species, *T. clevelandii*, which, however, has now been transferred to the genus *Platysiphonia* (Papenfuss, 1944, p. 206). Thus, there are only two species in the genus *Tænioma* and opinion is divided on the separation of these two. Bornet (1892) included *T. macrourum* Thuret under *T. perpusillum* J. Ag., and this has been accepted by a number of workers (De Toni, 1900; Howe in Thompson, 1910; Boergesen, 1919, 1930).

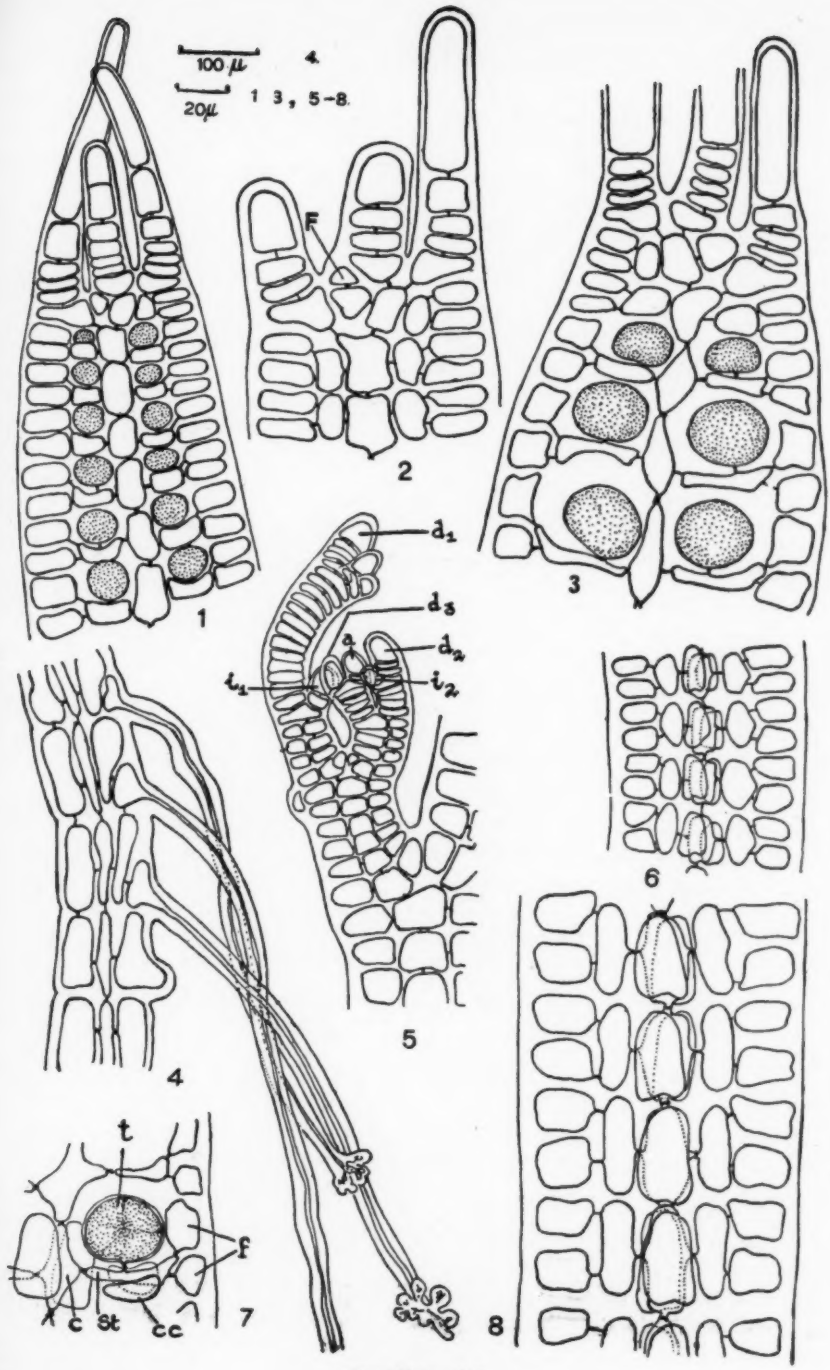
Falkenberg (1901) and Schiffner (1931) however, have kept the two separate. Recently, Papenfuss (1944) made an intensive study of *T. perpusillum* and maintained the separation of the two species (see also Tseng, 1944).

Polysiphonia nana was described by Kuetzing in 1863 and has been considered by Falkenberg (1901) and Boergesen (1919) as a synonym of *T. perpusillum*. Papenfuss (1952) examined Kuetzing's type material and transferred the species to *Tænioma* as *T. nanum* (Kuetz.) Papenfuss. He also considered *T. macrourum* and *T. nanum* identical and on the basis of priority accepted *T. nanum* as the valid binomial. Papenfuss (1944, 1952) thus recognises two species, *T. perpusillum* and *T. nanum*.

Papenfuss (1944) also gives the complete geographical distribution of these two species. It is evident that the genus has not so far been reported from India. The nearest known geographical situation is the Dirk Hartog Island (W. Australia) in the Indian Ocean and Arniston and Port Elizabeth in South Africa. The writers collected good material of this genus growing on *Laurencia* from Dwarka on the west coast of India. The material contained both the species and these are described here.

T. perpusillum

The Indian form is somewhat more robust than that described by Papenfuss. The prostrate creeping axis is anchored to the host plant by means of non-septate rhizoids (Text-Fig. 4). The erect secondary axes are produced on the dorsal side. Both the prostrate and erect axes are strictly monopodial



TEXT-FIGS. 1-8

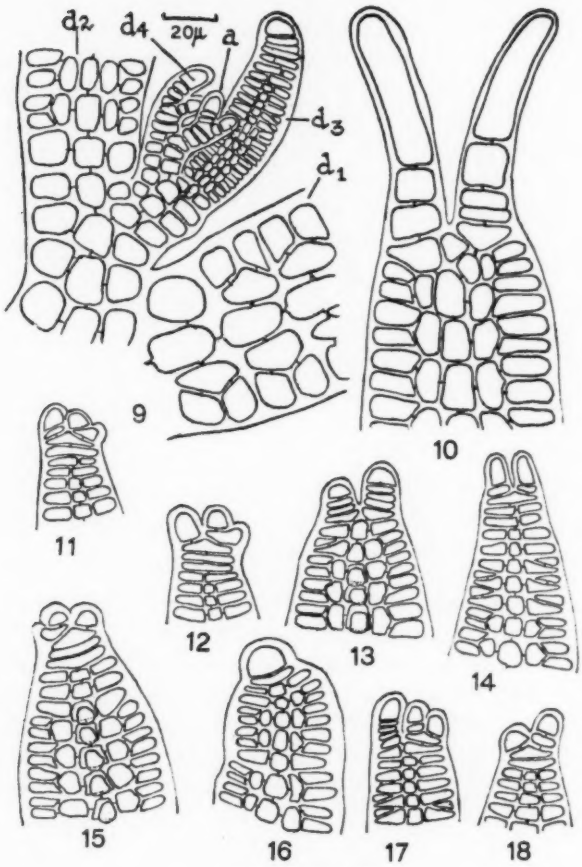
and growth is by means of an apical cell. This divides transversely and cuts off disc-shaped segments on the lower side. The indeterminate axes are polysiphonous, each segment having a central cell and four pericentral cells. The rhizoids arise as outgrowths from the ventral pericentral cells of the prostrate axes. Prostrate axes are 80–125 μ broad in the younger portions and 110–150 μ broad in the oldest portions.

The erect axes are arranged alternately at intervals of 4–8 segments on the dorsal side of the prostrate portion. In their turn, they give rise to alternate determinate laterals, starting from the 3–6 segments from the base and at intervals of 2–7 segments. In marked contrast to the indeterminate axes which are cylindrical, the determinate branches are flat and five cells in width except for the basal segments. Each determinate branch produces three, rarely two, hairs at the tip. With the initiation of these hairs, segment formation in the determinate branches ceases.

Even while quite young and only 2–3 segments long, each determinate branch initiates an indeterminate branch on the adaxial side (Text-Fig. 9). This in turn gives rise to a determinate branch of higher order which once again forms an indeterminate lateral, and so on repeatedly over and over again, so that a branch of one type gives rise always to a higher order branch of the opposite type. As a result of this frequent repetition of branching, the younger portions of the erect branches have a characteristically clustered appearance.

Branching in the prostrate and erect axes is exogenous, similar to that seen in *Polysiphonia* and other Rhodomelaceæ. The formation of a branch is initiated by an oblique division of the apical cell so that the segment formed is higher on one side. A protuberance is then formed on this higher side and this is cut off as the branch initial. Further divisions of this branch initial are transverse, giving rise to a monosiphonous filament whose segments then undergo division. The indeterminate branches produced adaxially on the determinate laterals are also likewise exogenous in origin. The erect axes as a rule produce determinate branches. The prostrate portion always gives rise to indeterminate axes.

The determinate laterals are formed alternately on the erect axes at intervals of 2–7 segments. As already stated, while only 2–3 segments long they produce exogenously indeterminate branches on the adaxial side (Text-Fig. 9). After production of these adaxial branches the apical cell of the determinate branch divides repeatedly to produce 15–23 segments. At this stage, the apical cell divides successively twice by oblique walls so that the determinate branch is topped by a set of three initials which



TEXT-FIGS. 9–18

grow out into three monosiphonous hairs, situated at the tip of the branch (Text-Figs. 1–3). Not infrequently, the apical cell divides only once by an oblique wall. Then only two hair initials instead of three are formed and the determinate branch becomes crowned by two hairs (Text-Fig. 10). Very rarely, perhaps due to injury or other causes, even this division does not take place and we have the determinate branch crowned by a single apical cell (Text-Fig. 16).

Further segment formation in the determinate laterals ceases with the production of the hairs. Intercalary divisions of the basal cells in these hairs has not been observed (see also Papenfuss, 1944, p. 197).

After the formation of the hair initials, the other cells of the determinate branches undergo further development. Pericentral cells are produced and their formation follows the usual pattern in the Delesseriaceæ; *i.e.*, the two lateral pericentral cells are formed first. These two are not formed simultaneously. The two transverse pericentral cells are cut off later, after both the lateral pericentral cells have been formed. The transverse pericentral cells do not divide further. Each of the lateral pericentral cells cuts off two flanking cells, the upper flanking cell being always formed first (Text-Figs. 14, 17). Thus, the mature determinate branches are flat structures, five cells wide, are one layer thick at the margins and three cells thick at the midrib (Text-Figs. 6, 8). Fully mature determinate branches are 65–125 μ broad. The two hair-forming segments at the tip of the branch also divide in a fashion similar to that of other segments, but in the distal segment the lateral pericentral cell opposite to the attachment of the hair does not form flanking cells (Text-Figs. 1, 3). Rarely, however, it does divide once to form an upper flanking cell (Text-Fig. 2). Thus, in the construction of the determinate branches *Tænioma* conforms to the general pattern seen in the Delesseriaceæ. The formation of lateral organs before pericentral cell formation resembles the pattern of the Rhodomelaceæ.

The material showed abundant tetrasporangia. The development of the tetrasporangia is similar to that described by Thompson (1910) and Papenfuss (1944). The tetrasporangia are formed by the lateral pericentral cells of the determinate branches and in regular acropetal succession (Text-Figs. 1, 3). Each lateral pericentral cell of the fertile segment functions as a tetrasporangium-mother cell and divides by a concave transverse wall (Text-Fig. 20). Of the two cells thus formed, the upper cell is the tetrasporangium and the lower the stalk cell. The stalk cell remains attached to the corresponding flank cells, while the tetrasporangium is connected only to the stalk cell (Text-Figs. 1, 3, 7). The tetrasporangium enlarges and undergoes division into four tetrahedral spores. The stalk cell cuts off two cells, the cover cells, one on each side (Text-Fig. 7, *cc*). These cover cells do not divide further or even enlarge so that the tetrasporangium remains exposed throughout. The formation of cover cells subsequent to the initiation of the tetrasporangium conforms to the general pattern in the Delesseriaceæ. Fertile determinate branches become distended due to the enlargement of the tetrasporangia and measure from 100–150 μ in width. In other words, they widen to about 1½ times their original size.

Sexual plants were not seen in the material.

In the details of development and morphology, the writers' observations are in agreement with those of Papenfuss (*loc. cit.*).

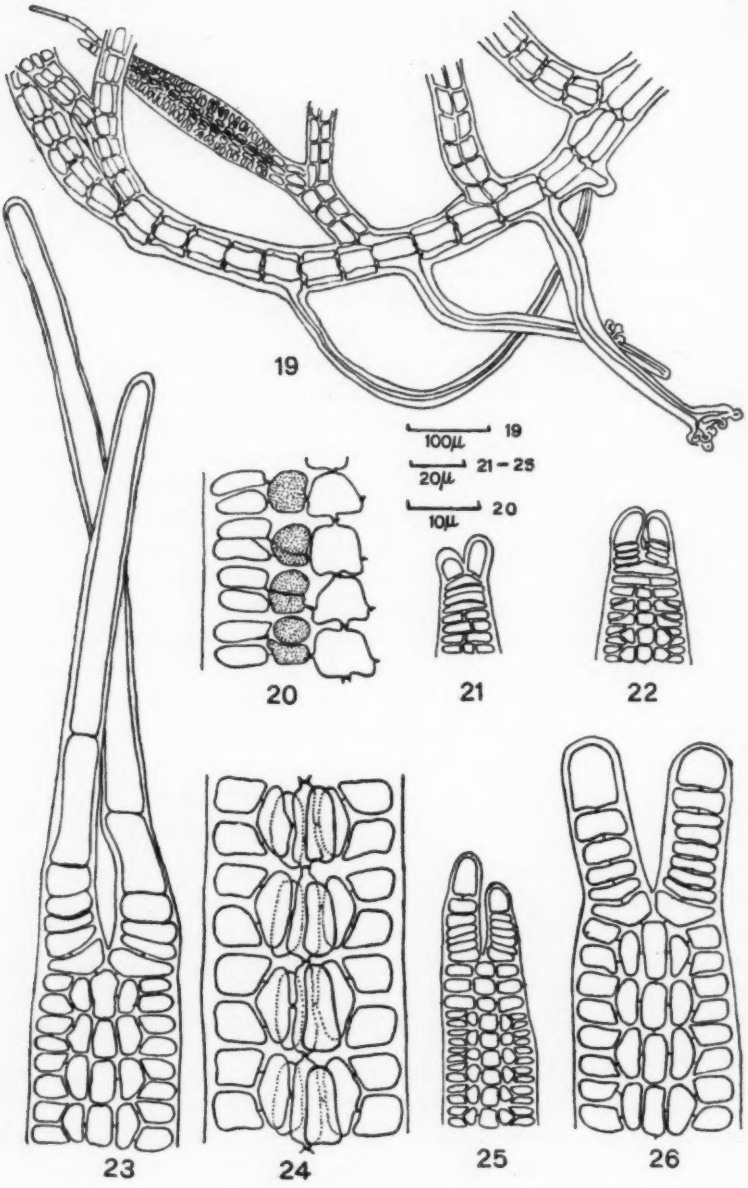
T. nanum

Only a few plants of this species were found intermixed with *T. perpusillum*. The plants are lighter in colour than those of *T. perpusillum*. The general habit is the same in the two species. The prostrate creeping axis is anchored to the host by non-septate rhizoids arising from the ventral pericentral cells. The tip alone is decumbent (Text-Fig. 19). The erect secondary indeterminate axes, similarly, are borne on the dorsal side at intervals of two to nine segments. Prostrate axes are 45 to 60 μ broad in younger parts and 60 to 100 μ in the old portions. Erect axes are 35 to 55 μ broad at the base. The construction plan of these axes is the same as in *T. perpusillum*, each segment having a central and four pericentral cells. The determinate branches are produced laterally on the erect axes starting from the fifth to the eighth segment from the base and at intervals of three to seven segments. As in *T. perpusillum*, the determinate laterals are flat, five cells in width and three cells thick in the middle (Text-Fig. 24). Pericentral cell formation in the segments is also quite similar (Text-Figs. 21, 22). Hair initiation takes place when the branch is 15–20 segments long. The apical cell divides once in an oblique fashion and the segment thus formed develops a hair on the higher side in true exogenous fashion. The apical cell grows out into the second hair (Text-Figs. 21, 22). Unlike in *T. perpusillum*, the number of hairs in this species seems very constant—there being always only two hairs, never three or one. Fully mature determinate branches are 50 to 95 μ broad.

While quite young and only two to three segments long the determinate branch forms an indeterminate branch initial on the adaxial side. This indeterminate branch develops a determinate lateral of the higher order and so on, just as in *T. perpusillum*. But while in the latter species there is a clustering at the tips owing to the frequency of this process, here the interval between the formation of successive branches is greater, leading to a general laxity in the branching. It was noted, however, that almost all the determinate branches produced adaxial branches near their base and that their production was not rare.

Conclusion

Tænioma is now represented by two species, *T. perpusillum* J. Agardh, 1863, and *T. nanum* (Kuetzing) Papenfuss (1952) (= *T. macrourum* Thuret in Bornet and Thuret, 1876). Bornet (1892), soon after the creation of



TEXT-FIGS. 19–26

T. macrourum reduced it to synonymy with *T. perpusillum* and this has been accepted by many workers. Falkenberg (1901) and following him Schiffner (1931) have, however, retained the two species distinct. The reasons which Falkenberg adduces for his stand are no longer tenable (see also Papenfuss, 1944). Recently Papenfuss (1944) (see also Tseng, 1944) has gone into this problem more thoroughly. He retains the two species distinct on two grounds. (1) In *T. perpusillum* the determinate branches invariably end in three hairs while in *T. nanum* (as *T. macrourum*) they end in two. (2) In *T. perpusillum* determinate branches form regularly adaxial indeterminate branches near the base while in *T. nanum* such branches are rare. Hence, the thallus is more profusely branched in *T. perpusillum* than in *T. nanum*.

The writers are in agreement with Papenfuss on the separation of the two species. Their present study confirms Papenfuss's observations on the two species. They found it easy to distinguish between the two species as the branching is much sparser in *T. nanum* than in *T. perpusillum*. Further, the prostrate and erect axes in the former species are also more slender. They have observed that in *T. perpusillum* the terminal hairs are usually three in number; but occasionally they have also seen two hairs. In *T. nanum* the hairs are always two in number and no case of formation of three hairs has been observed. Their other observations on the general features of thallus organisation are in full agreement with those of Papenfuss.

Summary

Both the known species of *Tænioma*, *T. perpusillum* J. Ag. and *T. nanum* (Kuetz.) Papenfuss, are here reported for the first time from India. General features of thallus organisation in both species are described in detail. The development of tetrasporangia in T. *Perpusillum* is described. In general, the writers' observations are in agreement with those of Papenfuss. The taxonomy of the genus is discussed and the authors agree with Papenfuss in keeping the two species distinct.

The authors are grateful to Prof. T. S. Sadasivan, F.A.SC., F.N.I., for kind facilities and encouragement.

References

Agardh, J. G. .. "Nya alger från Mexico," *Öfvers. Vetens.-Akad. Förh., Stokh.*, 1848, **4**, 5–17.

——— .. *Species, genera et ordines algarum*, 1863, **2** (3), 710–1291, Lund.

Boergesen, F. .. "The marine algæ of the Danish West Indies, 2, Rhodophyceæ (5)," *Dansk Bot. Arkiv.*, 1919, **3** (le), 305–68.

Boergesen, F. .. "Marine algæ from the Canary Islands, 3, Rhodophyceæ, Pt. III, Ceramiales," *Biol. Medd. Kbh.*, 1930, **9** (1), 393–536.

Bornet, E. .. "Les algues de P.K.A. Schousboe, récoltées au Maroc and dans la Méditerranée de 1815 à 1829," *Mém., Soc. nat. Sci. Cherbourg*, 1892, **28**, 165–376.

—— and Thuret, G. .. *Notes Algologiques, Paris*, 1876, **1**, xx + 70.

De Toni, J. B. .. *Sylloge Algarum*, Patavii, 1900, **4**, 1973 pp.

———— .. *Ibid.*, Patavii, 1924, **6**, 767 pp.

Falkenberg, P. .. "Die Rhodomelaceen des Golfes von Neapel und der angrenzenden Meeresabschnitte," *Fauna und Flora des Golfes von Neapel*, 1901, Monogr. **26**, 1–754.

Farlow, W. G. .. "On some algæ new to the United States," *Proc. Amer. Acad. Arts Sci.*, 1877, **12**, 235–45.

Kuetzing, F. T. .. *Tabulæ Phycologicæ.*, 1863, **13**, Nordhausen.

Papenfuss, G. F. .. "Structure and taxonomy of *Tænioma*, including a discussion on the phylogeny of the Ceramiales," *Madroño, S. Francisco*, 1944, **7** (7), 193–214.

———— .. "Notes on South African Marine Algæ, III," *J. Afr. Bot.*, 1951 (published in 1952), **17**, 167–88.

Schiffner, V. .. "Neue und bemerkenswerte Meeresalgen," *Hedwigia*, 1931, **71**, 139–205.

———— .. "Meeresalgen aus Süd-Dalmatien, gesammelt von Franz Berger," *Öster. bot. Z.*, 1933, **82**, 283–304.

Thompson, E. I. .. "The morphology of *Tænioma*," *Bull. Torrey bot. Cl.*, 1910, **37**, 97–106.

Tseng, C. K. .. "Notes on the algal genus *Tænioma*," *Madroño, S. Francisco*, 1944, **7** (7), 215–26.

EXPLANATION OF TEXT-FIGURES

TEXT-FIGS. 1–8.—*Tænioma perpusillum.* Figs. 1, 3. Determinate branches showing three terminal hairs and tetrasporangia. Fig. 2. Tip of determinate branch showing production of an extra flanking cell (F) by the distal hair-forming segment. Fig. 4. Portion of prostrate axis showing rhizoids. Fig. 5. Apical portion of indeterminate branch showing two young determinate branches (d_1, d_2) each of which in turn has initiated indeterminate branches (i_1, i_2) on the adaxial side. The stem apex (*a*) has also initiated a determinate branch of the 3rd order (d_3) on the left. The oldest determinate branch (d_1), on the left, is in an early stage of hair formation. Figs. 6, 8. Parts of young and mature determinate branches showing the structure. Fig. 7. Part of segment of a determinate branch showing: (1) the central cell (*c*), (2) tetrasporangium (*t*), (3) the stalk cell (*s*), (4) the two cover cells (*cc*), and (5) the two flanking cells (*f*).

TEXT-FIGS. 9–18.—*T. perpusillum.* Fig. 9. Formation of adaxial branch by a determinate-lateral. d_1, d_2, d_3, d_4, d_5 denote determinate branches of the corresponding order. Fig. 10. Tip of determinate branch with two hairs instead of the normal three. Figs. 11, 12, 15, 17. Stages in hair initiation by determinate branches ending in three hairs. In Fig. 15 the transverse pericentral cells are also shown. Figs. 13, 14, 18. The same in determinate branches with two hairs.

In Fig. 13 transverse pericentral cells are also shown. Fig. 17. A determinate branch crowned by a single apical cell.

TEXT-FIGS. 19, 21–26.—*T. nanum.* Fig. 19. Habit showing prostrate and erect axes, rhizoids and a determinate branch. Figs. 21, 22, 25. Tips of young determinate branches showing hair formation. Fig. 23. Tip of the mature determinate branch with two hairs. Fig. 24. Part of a mature determinate branch to show organisation. Fig. 26. Upper part of mature determinate branch. TEXT-FIG. 20.—*T. perpusillum.* Part of young fertile determinate branch showing stages in the formation of tetrasporangia. Note concave wall in the lateral pericentral cells.

978-57. Printed at The Bangalore Press, Bangalore City, by C. Vasudeva Rao, Superintendent and Published by The Indian Academy of Sciences, Bangalore.

THE DEVELOPMENT OF THE CHONDROCRANIUM IN *TRICHOPODUS TRICHOPTERUS* (Pall.)

By V. B. Marathe and D. V. Bal, F.A.Sc.

(*Department of Zoology, The Institute of Science, Bombay*)

Received April 12, 1957

CONTENTS

	Page
Introduction	347
Material and Methods	347
Observations	348
Discussion	366
Summary	373
References	374
Key to Abbreviations	375

Introduction

Very little work has been done on the development of fish skulls in India. In view of this, the present investigation was undertaken in order to examine the development of the chondrocranium in a fresh-water fish—*Trichopodus trichopterus* (Pall.), belonging to the family Anabantidæ. As the fish breeds easily in the laboratory, it was possible to collect successive stages in development in order to get a closely graded series.

Material and Methods

The fish bred on Saturday, the 24th January 1954, and the female was removed immediately after. The eggs hatched the next day at about 4-30 p.m. and the first batch of embryos was fixed 20 hours after hatching. The successive stages were fixed at frequent intervals to give a closely graded range. Out of 80 stages fixed only 10 stages have been described in this paper.

The larval stages were fixed in picroformol and acetic-alcohol. The serial sections of various stages were cut at 10 micra and stained mainly with Delafield's hæmatoxylin. No counterstain was used. The stages in which the bones had already developed, were decalcified in a solution of

one part of concentrated nitric acid in hundred parts of 70 per cent alcohol. Bulk stained preparations were made by van Wijhe's (1902) technique for the study of the development of the chondrocranium. Methylene blue gave better results than Victoria or Toluidin blue.

Wax model reconstructions were prepared in several cases from series of transverse sections. Graphic reconstructions were also made for studying the chondrocranium.

OBSERVATIONS

Stage 1 (37½ hours after hatching. Total length 2·4 mm.) (Fig. 1).—The trabecular rudiments (Fig. 1, *tc*) are appearing as independent rod-like procartilages, placed ventrally in the orbito-temporal region.

The notochord (*n*) is distinctly seen. On either side of the notochord are noticed plate-like procartilaginous parachordals (Fig. 1, *pc*). They are not so distinct as the trabeculæ. The auditory sacs are formed but the capsular cartilages are not laid down and concentration of mesenchymatous cells is seen in this region.

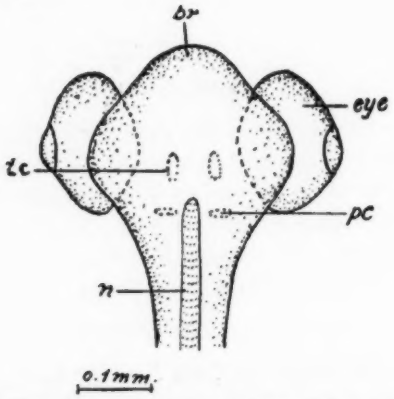


FIG. 1. Dorsal view of the brain and eyes of *Trichopodus trichopterus* (Pall.). Embryo 2·4 mm. long (model), stage 1.

The visceral skeleton is not yet chondrified. Accumulation of mesenchyme cells can be noticed in this region.

Stage 2 (40½ hours after hatching. Total length 2·5 mm.) (Figs. 2 and 3).—The cartilaginous trabeculæ (Fig. 2, *tc*) are better formed at this stage. They unite anteriorly to form a thin ethmoid plate (*ep*) which is about 60 micra in length. Posteriorly the trabeculæ unite with the anterior ends of the parachordals thus enclosing a large hypophysial fenestra (*hf*). The

notochord (*n*) extends anteriorly into the hypophysial fenestra The parachordals (Fig. 2, *pc*) are broader than the trabeculæ and lie on either side of the notochord. The rudiments of the auditory cartilages (*ac*) with well-developed anterior ends have appeared and they form the floor for the auditory vesicles. Each parachordal (*pc*) is united with the auditory cartilage of its side by means of a broad anterior basicapsular commissure (*abc*) and a small posterior basivestibular commissure (*bvc*). A small basicapsular fenestra (*bcf*) is enclosed between these two commissures. The branches of the trigeminal and facial nerves pass in front of the anterior basicapsular commissure. No side wall or cartilaginous roof is formed for the brain in this stage. The rudiments of the occipital arches (*oa*) are noticed on the posterior side of the parachordals.

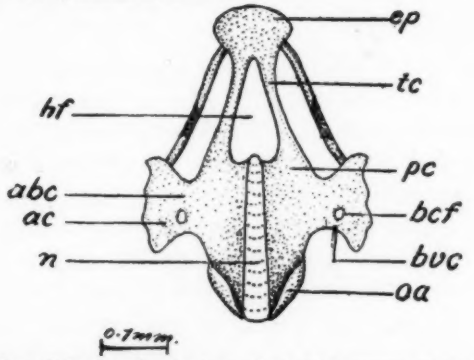


FIG. 2. Dorsal view of the chondrocranium of *T. trichopterus*, stage 2. Total length of larva 2·5 mm. (model). The hypobranchial arches are not shown.

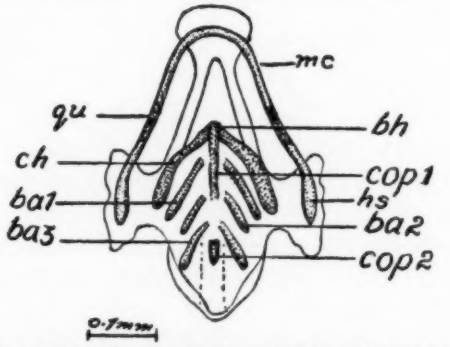


FIG. 3. Ventral view of the visceral arches of stage 8 (model).

The chondrification of some of the visceral arch elements (Fig. 3) has taken place. The two rami of Meckel's cartilage (*mc*) are united anteriorly to form a symphysis.

The pterygoquadrate is represented only by its quadrate portion (Fig. 3, *qu*). Posteriorly the quadrate is in contact with the lower portion of the hyosymplectic cartilage (*hs*). The hyosymplectic articulates with the ventral side of the auditory cartilage and functions as the suspensorium for both the jaws. The basihyal (*bh*) is continuous posteriorly with the basibranchial. It does not extend anteriorly beyond the ceratohyal (*ch*) in this stage. The basibranchials have united to form two copulæ, the anterior or the first copula (*cop* 1) continuous with the basihyal and the posterior one or second copula (*cop* 2), situated behind the first copula with a gap in between. The first copula extends behind up to the second branchial arch in this stage. The first three arches (*ba* 1–3) have appeared in this stage in the form of cartilaginous bars situated on either side of the copula. They are not differentiated into the various branchial elements. The branchial arches ventrally do not reach the copula.

Stage 3 (50 hours after hatching. Total length 2·8 mm.).—Except that the trabeculæ are more distinctly seen in this stage no new elements are added in the ethmoid region. The parachordals have increased in width. The basicapsular fenestra has also increased in size.

The pterygoquadrate bar has increased in width and extends slightly anteriorly. It is independent of Meckel's cartilage in this stage. The hyoid cornu is well developed and it unites dorsally with the hyosymplectic cartilage through a small procartilaginous stylohyal measuring about 10 micra.

The fourth branchial bar appears in this stage but does not reach the second copula ventrally. The first copula extends up to the third branchial arch. A pharyngobranchial is seen on either side but it is not connected to any of the branchial bars.

Stage 4 (55 hours after hatching. Total length 3 mm.).—The ethmoid plate has increased in width. The trabeculæ though slightly reduced in thickness are continuous behind with the parachordals.

The occipital arches extend anteriorly but do not reach the auditory region. The rudiments of the auditory capsular roof and side walls are making their appearance.

The stylohyal which was noticed as a small procartilaginous piece in the earlier stage now forms a broad cartilaginous structure, connecting the ceratohyal and the hyosymplectic.

The fifth arch has appeared measuring 40 micra.

Stage 5 (64 hours after hatching. Total length 3·0 mm.).—There is not much advance in this stage over the previous one excepting for a general

growth in all the cartilages present. The head vein is distinctly seen between the parachordal and the auditory capsule.

Stage 6 (69 hours after hatching. Total length 3·2 mm.).—The ethmoid region extends approximately between 80 and 90 micra. The basivestibular commissure has slightly increased in thickness.

The second copula supporting the fourth branchial arch has slightly increased in length.

Stage 7 (98½ hours after hatching. Total length 3·5 mm.).—The ethmoid plate has increased in size. The lamina orbitonasalis is not yet chondrified. Part of the dorsal epiphysial cartilage which has just appeared, measures about 10 micra and is one layer in thickness. The orbital cartilage is formed and is continuous with the epiphysial cartilage. It extends about 30 micra in front and 80 micra behind the epiphysial cartilage. The orbital cartilage is broader anteriorly and lies between the ethmoid and auditory regions but does not reach them.

The fifth branchial arch extends posterolaterally for about 100 to 110 micra, and consists of a single piece of cartilage which is anteriorly cylindrical, posteriorly slightly broader and dorsoventrally compressed. The first pharyngobranchial appears for the first time as a small circular piece of cartilage, extending up to 10 micra. It does not unite with the first ceratobranchial. The second and the third pharyngobranchials are distinctly seen to unite with the second and third ceratobranchials respectively. They seem to arise from the pharyngobranchial of the earlier stages.

Stage 8 (16th day after hatching. Total length 4·0 mm.) (Figs. 4–7).—The ethmoid plate (Figs. 4 and 5, *ep*) is rounded anteriorly as in previous stages. The medial nasal septum (Fig. 4, *ns*) is appearing. The trabeculæ have united to form the trabecula communis (*tcom*) thus reducing the extent of the hypophysial fenestra (*hf*). They are continuous posteriorly with the basal plate. The lamina orbitonasalis is noticed in one section. The orbital cartilage is well formed and unites with the auditory capsule by means of the post-orbital process, not seen in the earlier stages. The epiphysial cartilage (*epb*) has taken a definite shape with an anterior bend in the middle. The auditory capsule (*ac*) shows slight advance over the previous stage and its roof is being formed. The trigeminofacialis chamber (Fig. 5, *tfc*) is situated in front of the basicapsular fenestra (*bcf*) and is bounded anteriorly by the lateral commissure (*lc*). The pro-otic and the post-palatine processes forming the lateral commissure cannot be made out as arising independently from different parts of the cranium either in this stage or in the earlier stages examined.

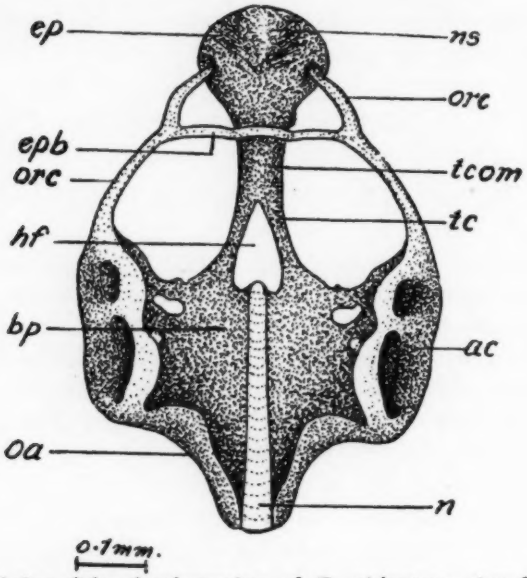


FIG. 4. Dorsal view of the chondrocranium of *T. trichopterus*, stage 8. Total length of larva 4·0 mm. (model). The visceral arches are not shown.

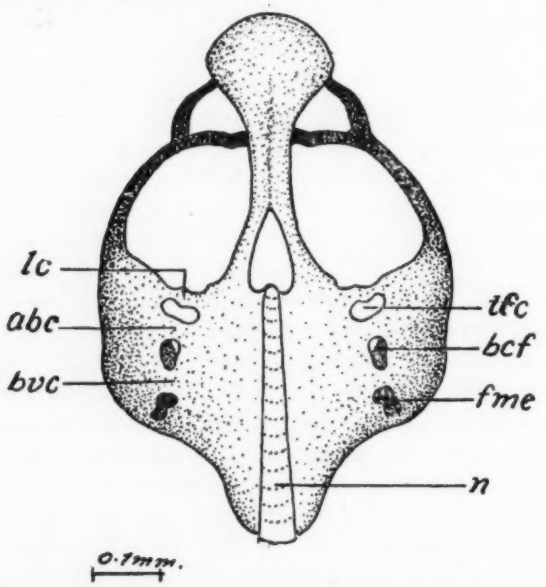


FIG. 5. Ventral view of the chondrocranium of stage 8 (model).

The synotic tectum is not formed in this stage. The occipital arches (Fig. 4, *oa*) are well formed and extend anterolaterally to join the hind end of the auditory capsules. The metotic foramen (Fig. 5, *fme*) is formed through which the IXth and Xth cranial nerves pass to the exterior.

The hyosymplectic cartilage (Fig. 6, *hs*) has increased in size. It bears a small foramen (*fh*) for the hyomandibular branch of the facial nerve. The pterygoid process (*ptp*) of the pterygoquadrate cartilage extends anteriorly up to the ethmoid plate but does not articulate with it in this stage.

The broad and anteriorly rounded basihyal (Fig. 7, *bh*) continues behind into the first copula. The paired hypohyals (*hh*) are well defined and they unite with each other on the ventral side of the basihyal. The first copula after giving attachment to the first three pairs of branchial arches slightly bends ventrally and then extends posteriorly for over 20 micra below the second copula. The second copula (*cop* 2) is small and gives attachment mainly to the fourth and the fifth pairs of branchial arches.

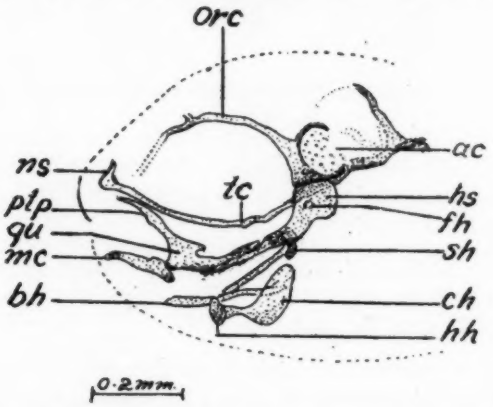


FIG. 6. Lateral view of the chondrocranium of stage 8 (van Wijhe's technique).

The first hypobranchial is distinct from the first ceratobranchial. The demarcation line between the epibranchials and the ceratobranchials cannot be made out at this stage. The last pharyngobranchial (*phb* 3 and 4) appears to be a combined one as it joins the third and fourth branchial arches, formed probably by the third and fourth pharyngobranchials.

Stage 9 (Total length 6·0 mm.) (Figs. 8–11).—The ethmoid plate (Fig. 8, *ep*) shows two small projections anterolaterally. The median nasal septum (*ns*) which arises in the anterior part of the ethmoid plate is well developed. Dorsolaterally it is united with the sphenoseptal commissure (*sphc*) of the

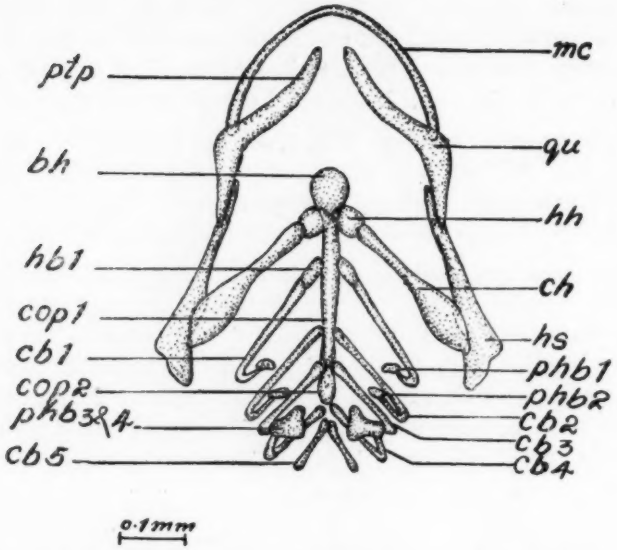


FIG. 7. Ventral view of the visceral arches of *T. trichopterus* of stage 8 (model).

orbital cartilage. The lamina orbitonasalis (Fig. 8, *lon*) is well developed and joins dorsally the orbital cartilage. The trabecula communis (*tcom*) has lost its connection posteriorly with the basal plate so that the hypophysial fenestra becomes obliterated.

The orbital cartilage (Fig. 8, *orc*) is quite prominent and develops the sphenoseptal commissure anteriorly. The epiphysial cartilage (*epb*) is broadened particularly in the middle.

The notochord is squeezed out of the basal plate (Figs. 8 and 9, *bp*) excepting in the posterior region of the cranium. Anteromedially the basal plate has a small notch which marks the posterior limit of the hypophysial fenestra. The auditory capsule is well developed and shows internally two septa—the septum semicircularis lateralis and the septum semicircularis posterius. The roof of the auditory capsule is completed and the synotic tectum (Fig. 8, *tsy*) connecting the two capsules dorsally is well developed. The lateral and the anterior basicapsular commissures are ossified and are no longer cartilaginous. The head vein and the orbital artery become enclosed between the inner and the outer lamellæ of the pro-otic bone, in a space termed pars jugularis of the trigeminofacialis chamber. This chamber is thus separated from the pars ganglionaris (V and VII ganglia) by the inner lamella of the pro-otic bone.

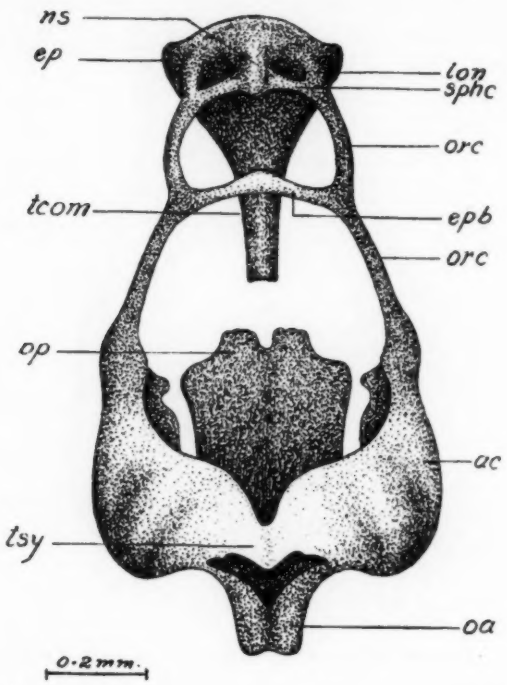


FIG. 8. Dorsal view of the chondrocranium of *T. trichopterus*, stage 9. Total length of larval form 6·0 mm. (model). The visceral arches and the ossified portions are not shown.

The basivestibular commissure (Fig. 14, *bvc*) is quite prominent and the head vein lies ventral to it. The occipital region is without a roof.

The pterygoid process (Figs. 10 and 11, *ptp*) articulates with the ventro-lateral side of the ethmoid plate. The quadrate part of the pterygoquadrate cartilage (Fig. 10, *qu*) is broad and gives articulation to Meckel's cartilage ventrally. Part of the hyosymplectic cartilage anterior to the opening of the hyomandibular foramen is ossified (Fig. 10, *hs*). The remaining elements of the hyoid arch (Fig. 11) are well developed.

The first copula (Fig. 11, *cop* 1) is continuous in front with the basihyal (*bh*). Immediately behind the basihyal, it enlarges into a large vertical plate and then divides into two parts, a dorsal and a ventral in the region of the first branchial arch (Fig. 11, *cop* 1). The dorsal part extends behind upto the second copula and the ventral runs posteriorly for a short distance. The first copula (*cop* 1) gives attachment to the first three pairs of branchial arches

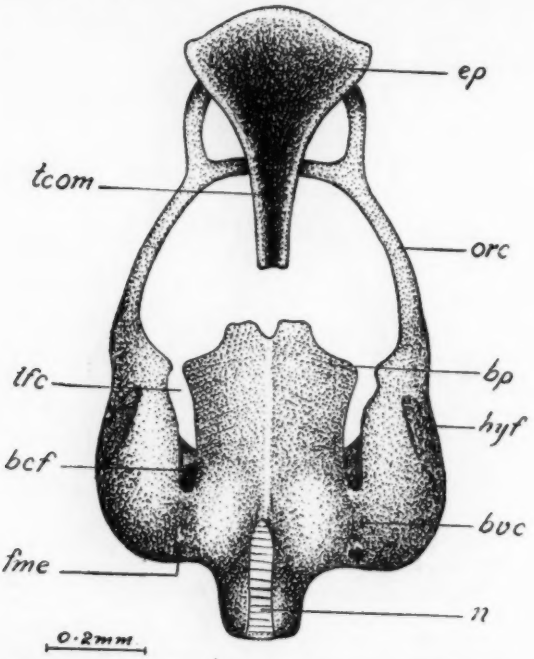


FIG. 9. Ventral view of the chondrocranium of stage 9 (model).

and the second to the remaining two. However, it is interesting to note that the third hypobranchial (*hb* 3) is connected with the second copula as well. Only the first epibranchial (*eb* 1) can be seen as an independent cartilage, as

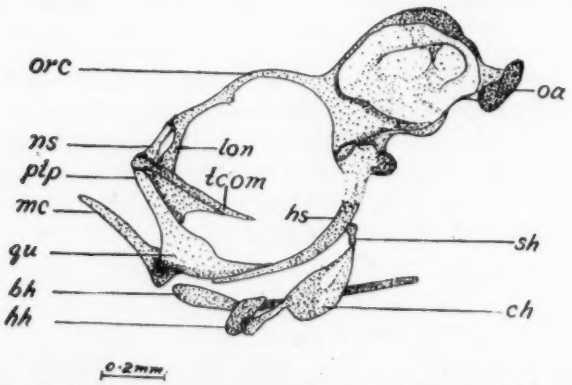


FIG. 10. Lateral view of the chondrocranium of *T. trichopterus* of stage 9 (van Wijhe's technique). Branchial arches are not shown.

the remaining epibranchials are not differentiated in this stage. The first three pharyngobranchials are clearly seen and they are supported by muscles

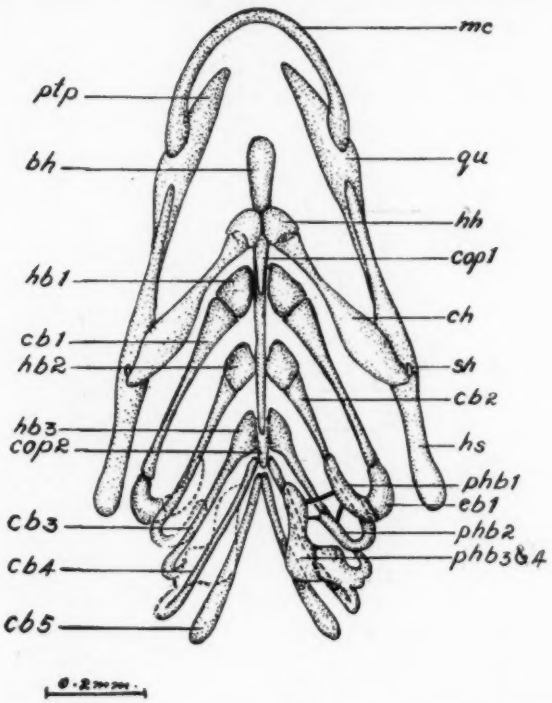


FIG. 11. Dorsal view of the visceral arches of *T. trichopterus*, stage 9 (model).

from the ventral wall of the auditory capsule. The first two pharyngobranchials (*phb* 1 and 2) are comparatively smaller than the third (*phb* 3 and 4) which is connected with the third and the fourth branchial arches. It appears to be formed by the fusion of the third and the fourth pharyngobranchials.

Stage 10 (Total length 11·5 mm.) (Figs. 12–18).—*Morphology of the fully formed chondrocranium.*—

(*a*) *Cranium*

1. *The ethmoid region* (Figs. 12–15).—This region is well developed and consists of a prominent ethmoid plate, a nasal septum and a pair of laminæ orbitonasales. A rostral cartilage is not noticed in this fish,

The ethmoid plate (*ep*) is round anteriorly and bears ventrally a shallow depression or groove; dorsally is seen a large nasal septum (*ns*) which is broader than in previous stages. It is connected posteriorly to the sphenoseptal commissure of the orbital cartilage. The lamina orbitonasalis (*lon.*) is in contact with the posterolateral side of the ethmoid plate and its dorsal end merges with the orbital cartilage. The olfactory foramen is bordered laterally by the lamina orbitonasalis and medially by the nasal septum.

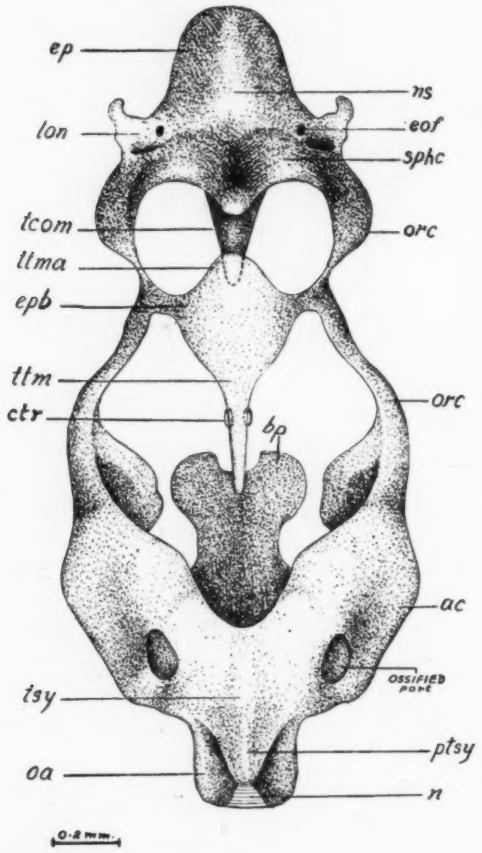


FIG. 12. Dorsal aspect of the chondrocranium of *T. trichopterus*, stage 10. Total length of larva 11·5 mm. (model). The visceral arches are not shown.

In this fish the brain extends very much anteriorly within the cranial cavity and hence the olfactory nerve after emerging out of the chondrocranium

through the olfactory foramen innervates immediately the olfactory sac. The foramen olfactorium advehens of *Salmo* (de Beer, 1937) is not noticed in this fish and hence the olfactory foramen may be called the foramen olfactorium evehens. This foramen is traversed by the orbitonasal artery

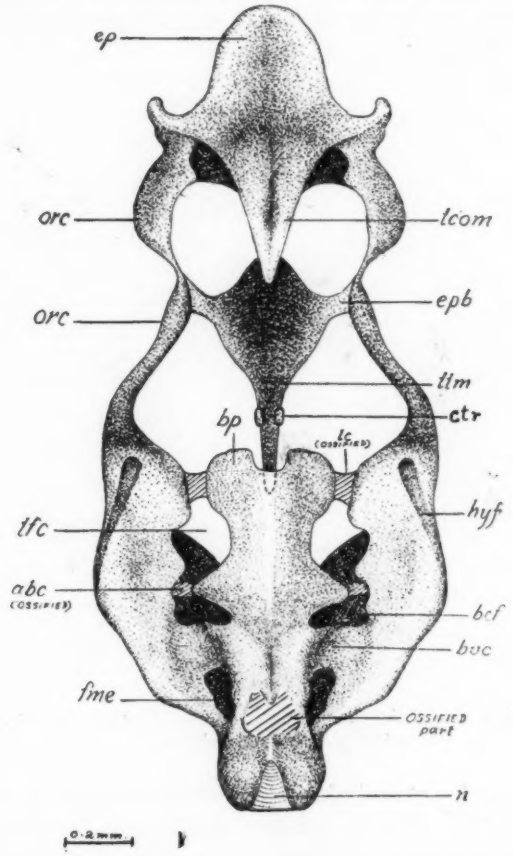


FIG. 13. Ventral view of the chondrocranium of stage 10 (model).

in addition to the olfactory nerve. The olfactory sac is not covered by any cartilage either dorsally or laterally. Even on the ventral side there is no direct cartilaginous support as the sac is placed at a much higher level than the ventral ethmoid plate.

2. *The orbito-temporal region* (Figs. 12–17).—Posterior to the ethmoid plate, the trabecula communis is seen (Figs. 12–14, *tcom*) running in a postero-

ventral direction. As pointed out in the previous stage it does not reach the basal plate, resulting in the obliteration of the hypophysial fenestra. It is quite broad in front and tapers behind. Ventrally the trabecula communis shows a distinct median groove running throughout its length and continues with the shallow depression of the ethmoid plate. This groove lodges the parasphenoid bone.

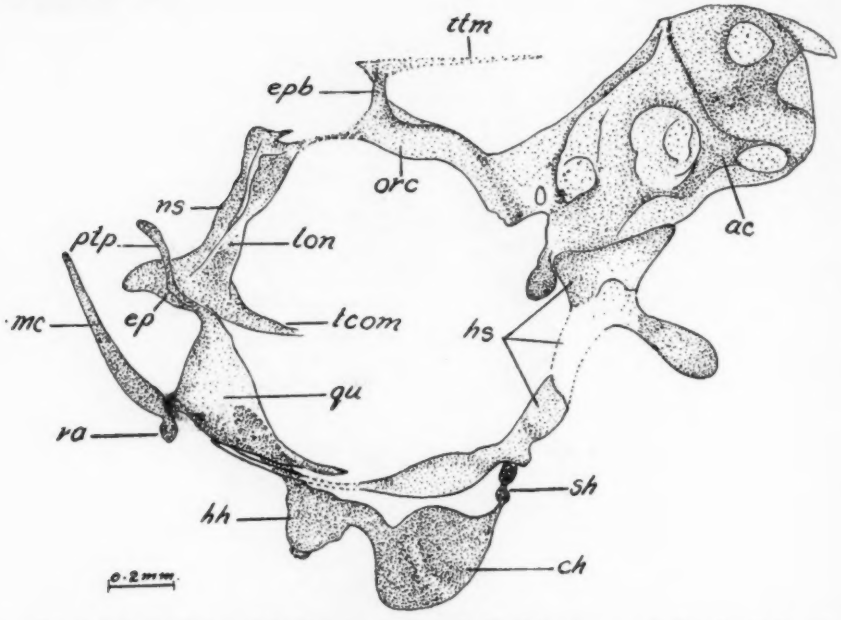


FIG. 14. Lateral view of the chondrocranium of *T. trichopterus*, total length 12·0 mm. (van Wijhe's technique). Branchial arches are not shown.

The well-developed and elongated orbital cartilage or tænia marginalis is seen extending between the auditory capsule and the ethmoid region. It joins the epiphysial cartilage near its middle, in front of which it is quite thin for a short distance and anteriorly it develops into a broad massive cartilage uniting in front with the lamina orbitonasalis and also with the nasal septum through its median sphenoseptal commissure. The sphenoseptal commissures from the two sides unite in the middle and form a prominent roof over the anterior region of the brain (Fig. 12, *sphc*). This roof has a distinct median groove with an arched portion on either side. Posteromedially the roof is produced into a small blunt process which does not reach the tænia tecti medialis anterior.

The epiphysial cartilage (Fig. 12, *epb*) is very well developed and is quite large. It extends anteriorly and posteriorly into tænia tecti medialis. The posterior extension is triangular in shape and forms a roof of the brain in this region.

A membranous inter-orbital septum is present and it generally limits the anterior and ventral extension of the brain in fishes. In *Trichopodus* it, however, limits the ventral extension of the brain. The septum is

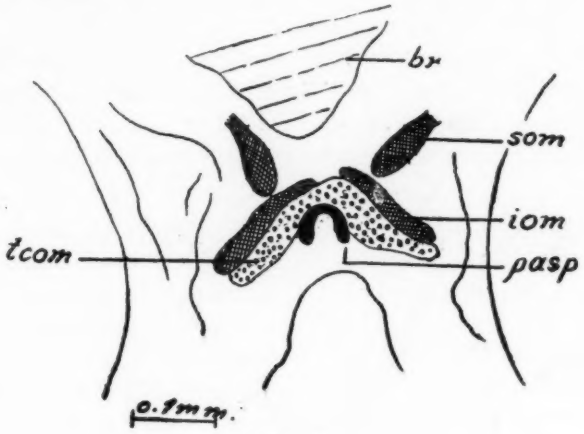


FIG. 15.

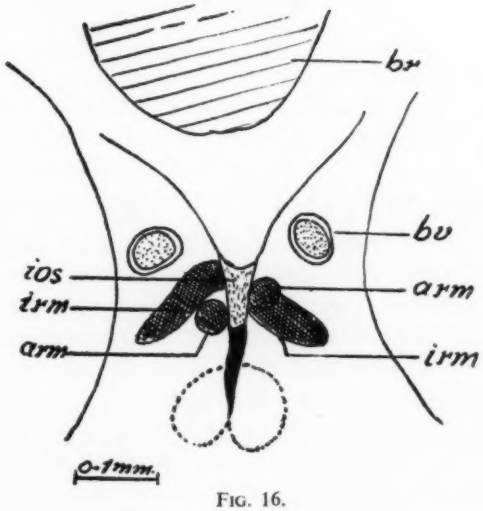


FIG. 16.

ventrally attached to the trabecula communis and behind it to the parasphenoid bone. Dorsally it is attached throughout its length to the membranous floor (dura mater) of the brain-cavity. Anteriorly the inter-orbital septum is quite thick and broad and is formed of connective tissue. It then gradually becomes laterally compressed into a narrow partition. Further behind, its lower part is reduced giving it a somewhat triangular appearance. In the posterior-most part, the septum though thin increases in height and joins the parasphenoid bone.

The oblique eye-muscles (Fig. 15, *som* and *iom*) are attached to the dorsal margin of the trabecula communis (*tcom*) even before the formation of the inter-orbital septum. The point of attachment of the superior oblique muscle is slightly anterior to that of the inferior. There is no anterior eye-muscle canal as in *Clupea* (Norman, 1926).

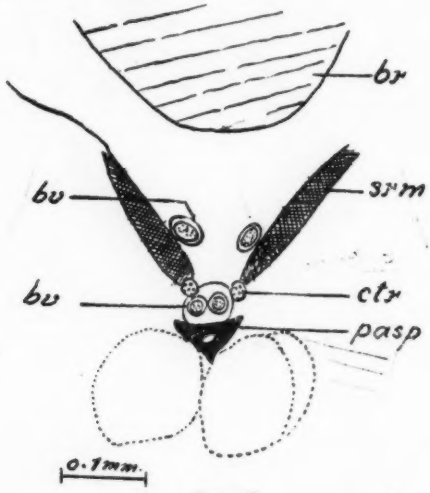


FIG. 17.

FIGS. 15–17. Transverse sections of *T. trichopterus*, stage 10.

The anterior and inferior recti-muscles join the inter-orbital septum (Fig. 16, *arm* and *irm*). The point of attachment of the inferior rectus muscle is situated dorsal to that of the anterior on one side and *vice versa* on the other. In addition the inferior rectus muscle is attached posteriorly to the thin wall of the brain-cavity.

The superior rectus muscle (Fig. 17, *srm*) runs beyond the point of attachment of the inferior rectus and is attached to a small rod-like cartilage (*ctr*).

This short cylindrical cartilage is not noticed in earlier stages. These paired cartilages together with the ventrally placed parasphenoid, form a small chamber, enclosing the internal carotid arteries. The superior rectus muscle after its attachment with the above cartilage extends behind for over 20 micra.

The posterior rectus muscle runs beyond the rod-like cartilage and is attached to the thin membranous ventral wall of the brain-cavity. This membranous wall also forms the roof of the chamber lodging the internal carotid arteries. In this region the parachordals are seen as small independent cartilaginous rods forming the lateral walls of the chamber. The two internal carotid arteries are separated from one another by a connective tissue septum.

An independent complete ring of sclerotic cartilage is developed around each eye.

Both lateral and anterior basicapsular commissures are ossified as noticed in the previous stage. A few cartilaginous cells are seen scattered inside the ossified commissures. The branches of the trigeminal nerve leave the cranium in front of the lateral commissure. The facial ganglion is distinct and is situated slightly ventral and medial to the head vein. The palatine nerve is also placed medial to the head vein but situated anterior to the other branches of the facial nerve. It starts from the brain as an independent branch and leaves the cranium along with the branches of the trigeminal nerve in front of the ossified lateral commissure.

3. *Auditory capsules* (Figs. 12–14).—The auditory capsules (*ac*) are separated from the brain-cavity by a thin membrane as in *Salmo* (de Beer, 1937) and *Gambusia* (Ramaswami, 1945). The auditory sacs are surrounded by cartilage and the outer cartilaginous wall of the lateral and posterior semicircular canals is partly ossified.

This ossified region has been noticed as a definite cartilage in the previous stage (6 mm. larva). The abovementioned two canals are raised and hence their boundaries can be clearly made out externally. Internally, the semicircular canals are separated from the brain-cavity by two cartilaginous septa—the septum semicircularis lateralis and the septum semicircularis posterius. The former septum is a prominent pillar-like cartilage, disposed slightly obliquely. The latter which separates the posterior semicircular canal is vertical and not prominent. The anterior semicircular canal is not separated from the brain-cavity by any septum. The auditory capsular cartilages extend dorsally as the tectum synoticum, covering the hind brain

(Fig. 12, *tsy*). Posteriorly, the tectum extends over the occipital region as a conical process (Fig. 12, *ptsy*). The head of the hyosymplectic cartilage fits in a groove on the ventral side below the bulge of the lateral semicircular canal (Fig. 13, *hyf*).

4. *The occipital region* (Fig. 12).—A pair of occipital cartilages (*oa*) is noticed in the posterior part of the chondrocranium and each one of them is continuous anteriorly with the corresponding auditory capsule. Posteriorly they are very much reduced and lie on the dorsolateral side of the notochord.

5. *The basal plate* (Figs. 12 and 13).—The basal plate (*bp*) is well developed and extends medially between the orbito-temporal and the occipital regions. Anteriorly it shows a distinct notch, marking the posterior boundary of the original hypophysial fenestra. The lateral and the anterior basicapsular commissures are ossified as already stated. The basivestibular commissure (Fig. 13, *bvc*) is well represented and the metotic foramen (*fme*) situated behind it serves as a common exit for the glossopharyngeal and vagus nerves. The metotic foramen is slightly ossified. Near its posterior end a median part of the basal plate is ossified as shown by a gap in the figure.

6. *The notochord.*—In this stage the notochord which is much reduced extends into the cranium for a short distance. Ventrally it is exposed throughout its length. Dorsally it is covered in front by the basal plate and the remaining part is visible between the occipital arch elements.

(*b*) *Visceral arches*

7. *The mandibular arch.*—The mandibular arch, consisting of an upper pterygoquadrate and a lower Meckel's cartilage is now fully formed.

(*i*) *The pterygoquadrate* (*the palato-quadrate*) (Fig. 14).—It consists of an extensive quadrate part (*qu*) giving articulation ventrally to Meckel's cartilage (*mc*). It has a posterior process extending over a ventral part of the hyosymplectic cartilage with which it does not unite. The pterygoid process (*ptp*) is a slender bent cartilaginous piece, running in front dorsal to the ethmoid plate. As there is no special ethmopalatine process it articulates directly with the ethmoid plate.

(*ii*) *Meckel's cartilage* (Fig. 14).—The rod-like Meckel's cartilage (*mc*) is disposed obliquely and extends anteriorly beyond the ethmoid plate. The anterior symphysis of Meckel's cartilages is ossified and is replaced by dentaries. Posteriorly Meckel's cartilage extends behind the point of articulation with the pterygoquadrate as a small knob-like retroarticular process (*ra*).

8. *The hyoid arch* (Figs. 14 and 18).—The well developed, rod-like, basihyal (Fig. 18, *bh*) extends anteriorly upto the level of the ethmoid region and continues behind with the basibranchial. The two hypohyals (Figs. 14 and 18, *hh*) are stout cartilages which partially encircle the basihyal. They are not perforated by the hyoidean artery nor are they delimited from the ceratohyals.

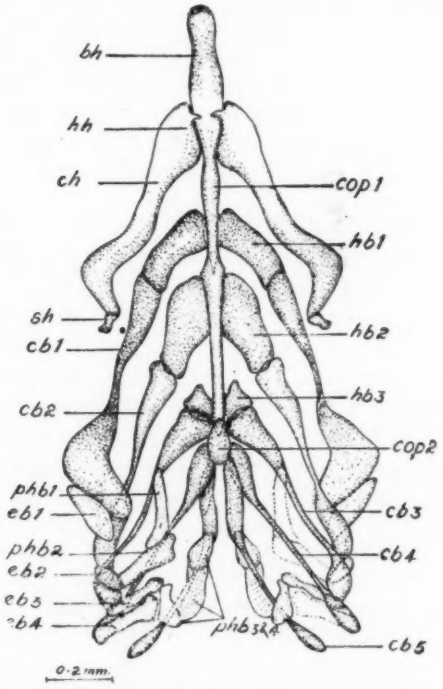


FIG. 18. Dorsal view of the hypobranchial skeleton of *T. trichopterus*, stage 10 (model). Hyomandibular and symplectic are not shown.

The ceratohyals (*ch*) are slightly bent, elongated cartilages, quite broad in their posterior half. They extend anteriorly below the hypohyals with which they are firmly united. The stylohyal (*sh*) is a small dumbel-shaped cartilage, connected dorsally to the hyosymplectic and ventrally to the ceratohyal.

Ossification has set in the hyosymplectic cartilage (Fig. 14, *hs*) in the region of the hyomandibular foramen. The head of the hyosymplectic abuts against the ventrolateral side of the chondrocranium in the auditory region and extends from the lateral commissure to the basicapsular fenestra.

A distinct rod-like cartilage arises from the upper part of the hyosymplectic and runs posteriorly to give attachment to the opercular bone.

The lower portion of the hyosymplectic is elongated and extends ventrally below the quadrate. Perichondral ossification is noticed in this region. It supports the stylohyal in a notch near its upper part.

9. *The branchial arches* (Fig. 18).—All the five pairs of branchial arches are present. The first three arches show all the elements namely the hypo-cerato-, epi-, and pharyngo-branchials. The fourth arch has a common hypo-ceratobranchial and the pharyngobranchials of the third and fourth arches are united. The fifth arch consists of only the ceratobranchials. Ossification has already set in and a major part of the cartilage from the middle part of the ceratobranchial is ossified.

The basibranchials unite to form the median copulæ, to which the hypobranchials or ceratobranchials are united. In this fish there are two distinct copulæ (*cop* 1 and *cop* 2) an anterior and a posterior. The anterior copula (*cop* 1) is very much elongated and gives attachment to the first three hypobranchials. As pointed out in the previous stage (6·0 mm. larva), the first copula consists of two parts a short ventral and an elongated dorsal piece. The posterior copula (*cop* 2) is short and supports the ceratobranchials of the third arch and the hypo-ceratobranchials of the fourth arch. The fifth arch (*cb* 5) is being ossified and is connected with the second copula through a ligamentous tissue. All the four epibranchials (*eb* 1–4) are well developed the second being the shortest.

The first two pharyngobranchials (*phb* 1 and 2) are rod-shaped and smaller in size than the common third and fourth pharyngobranchial (*phb* 3 and 4) which is ossified in the middle.

DISCUSSION

In fish the trabeculæ arise from the mesenchymatous plaques which are in contact with the epidermis and they are continuous with the mesenchyme of the mandibular arch (de Beer, 1931). A transverse commissure connects the two mandibular somites in *Salmo* (de Beer, 1931) which is absent in *Ophicephalus* (Srinivasachar, 1953). In *Trichopodus* the trabecular rudiments appear as rod-like procartilages in a 2·4 mm. larva and the mesenchymatous plaques described by de Beer or the mesenchymatous tracts recorded by Srinivasachar cannot be made out. The appearance of mesenchymatous tracts in the development of *Trichopodus* may have been skipped due to the shortened larval life.

The time of appearance of the trabeculæ in relation to that of parachordals varies in different teleosts. The trabeculæ appear to develop earlier than the parachordals in *Salmo* (Stohr, 1882), *Lepidosiren* and *Protopterus* (Agar, 1908). In *Sebastes* (Mackintosh, 1923) the trabeculæ are laid down as definite tracts of procartilage even when there is no trace of parachordals. The parachordals appear earlier than the trabeculæ in *Salmo* (de Beer, 1927), *Acipenser* (Sewertzoff, 1928) and *Polypterus* (Moy Thomas, 1933). In *Ophicephalus* (Srinivasachar, 1953) the two elements seem to develop simultaneously.

In the case of *Trichopodus* the trabeculæ seem to form slightly earlier than the parachordals, because the trabeculæ are better represented in a 2·4 mm. larva (Stage 1) than the parachordals.

In many teleosts the trabeculæ behind the ethmoid plate fuse medially to form a trabecula communis but posteriorly they are free and continuous with the parachordals, enclosing a small hypophysial fenestra. This type of cranium is called the tropitrabic cranium and generally possesses an inter-orbital septum as in *Syngnathus* (Kindred, 1921), *Clupea* (Wells, 1922), *Sebastes* (Mackintosh, 1923), *Anguilla* (Norman, 1926) and *Salmo* (de Beer, 1927).

In fishes like *Gasterosteus* (Swinnerton, 1902), *Gymnarchus* (Assheton, 1907), *Amiurus* (Kindred, 1919) and *Amia* (Pehrson, 1922) the cranium is of the platytrabic type where the trabeculæ remain apart and the inter-orbital septum is absent. In *Ophicephalus* (Srinivasachar, 1953) a trabecula communis is present but no inter-orbital septum is formed. It is also called the platytrabic type of cranium.

The cranium of *Trichopodus* belongs to the tropitrabic type as the trabecula communis as well as the inter-orbital septum are present.

Generally one or more fenestræ are present in the floor of the chondrocranium and they may or may not be closed by cartilage or bone at a later stage. In the platytrabic type of cranium there is a single large hypophysial fenestra and the notochord projects into it. In *Lepidosteus* (Veit, 1911) and *Amia* (Pehrson, 1922) this fenestra shows two broad sections, an anterior and a posterior.

In the tropitrabic cranium the hypophysial fenestra is present but it is smaller than in the platytrabic type. It may be divided into anterior and posterior compartments due to the widening of the bordering cartilage as in *Gasterosterus* (Swinnerton, 1902) and *Salmo* (de Beer, 1927).

In *Gymnarchus* (Assheton, 1907) and *Anguilla* (Norman, 1926) a narrow internal carotid foramen is separated off from the hypophysial fenestra.

In *Trichopodus* the hypophysial fenestra is small and triangular in outline. In later stages the fenestra is obliterated due to the absorption of the posterior part of trabeculæ, as noticed in *Gambusia* (Ramaswami, 1945) and *Ophicephalus* (Srinivasachar, 1953).

The laminæ orbitonasales arise as upgrowths from the posterolateral edges of the ethmoid plate and dorsally fuse with the anterior ends of the orbital cartilages or with the nasal septum. They form the outer bounding wall for the olfactory foramen. When the lamina orbitonasalis is broad and massive as in *Cyclopterous* (Uhlmann, 1921) and *Salmo* (de Beer, 1927) the olfactory foramen is comparatively reduced and when small the olfactory foramen is large as in *Anguilla* (Norman, 1926). This shows that the size of the olfactory foramen is determined by the size of the lamina orbitonasalis.

In Cyclopterus (Uhlmann, 1921) the lamina orbitonasalis seems to arise from two centres of chondrification, a ventral and a dorsal. Procartilage is formed between these two centres. Later, the two centres grow and fuse with each other. In *Syngnathus* (Kindred, 1921) the laminæ orbitonasales do not meet the ventral ethmoid plate and this is considered as a secondary modification. In *Trichopodus* the lamina orbitonasalis develops as an upgrowth from the posterolateral side of the ethmoid plate.

The foramen through which the olfactory nerve enters the nasal cavity may not be the same as the one through which the olfactory nerve leaves the cranial cavity (de Beer, 1937). The foramen leading the olfactory nerve out of the true cranial cavity is called foramen olfactorium evehens. It is bounded posteriorly by the preoptic root of the orbital cartilage, ventrally by the ethmoid plate, anteriorly by the nasal septum and dorsally by the sphenoseptal commissure.

The olfactory foramen in fishes like *Polypterus* (Moy Thomas, 1933), *Acipenser* (Sewertzoff, 1928), *Gadus* (de Beer, 1937) opens directly into the nasal cavity. But in other fishes such as *Salmo* (de Beer, 1927), *Lepidosteus* (Veit, 1911) and *Amia* (Pehrson, 1922), due to the backward shifting of the pre-optic root or due to the forward movement of the lamina orbitonasalis, the foramen olfactorium evehens no longer opens directly into the nasal cavity, but into an extracranial space the cavum orbitonasale. This space is also traversed by the nasal branch of the profundus nerve and the orbitonasal artery. The cavum orbitonasale opens anteriorly into the nasal cavity by a wide mouthed opening, the foramen olfactorium advehens, bounded laterally by the lamina orbitonasalis, medially by the nasal septum and ventrally by the ethmoid plate.

In *Ophicephalus* (Srinivasachar, 1953) the foramen olfactorium evehens and advehens are said to be confluent.

In *Trichopodus* the brain extends anteriorly and the olfactory nerve after emerging out of the cranium through the foramen olfactorium evehens directly innervates the olfactory sac. The foramen olfactorium advehens is therefore absent.

The orbital cartilage gives rise in front to a sphenoseptal commissure joining the nasal septum as noticed in *Gasterosteus* (Swinnerton, 1902), *Salmo* (de Beer, 1927) and *Gambusia* (Ramaswami, 1945). In *Syngnathus* (Kindred 1921) the orbital cartilages are practically non-existent, excepting for the anterior and posterior ends in connection with the lamina orbitonasalis and the auditory capsule respectively.

In *Trichopodus* orbital cartilages are complete and well developed. The sphenoseptal commissure is also present.

The manner in which the supra-orbital bars appear in different teleosts is not uniform. In *Clupea* (quoted by Norman, 1926) each bar arises as an outgrowth from the anterior end of the auditory capsule and eventually reaches the anterior part of the cranial roof. In *Gasterosteus* (Swinnerton, 1902) and *Cyclopterus* (Uhlmann, 1921) the orbital cartilages appear to be interconnected with the epiphysial bar. They soon unite anteriorly with the laminæ orbitonasales and posteriorly with the post-orbital process of the auditory capsules.

In *Amia* (Pehrson, 1922) a pair of small orbital cartilages is developed. Each one of them unites in front with a posterior process of the lamina orbitonasalis and behind with the auditory capsule. The epiphysial cartilage appears independently. In *Trichopodus* the orbital cartilages develop in association with the epiphysial bar, in a 3·5 mm. larva (Stage 7). In later stages they extend and join the laminæ orbitonasales and the nasal septum anteriorly and the auditory capsules posteriorly.

The lateral commissure in *Salmo* (de Beer, 1927) is formed by the union of two processes, the post-palatine process arising from the anterolateral side of the basal plate and the pro-otic process coming from the anterior side of the auditory capsule. According to Norman (1926) the lateral commissure seems to arise as a projection from the lower edge of the thickened anterior end of the auditory capsule. This projection grows inwards between the roots of the fifth and seventh cranial nerves, finally fusing with the anterior end of the basal plate.

In *Ophicephalus* (Srinivasachar, 1953) a massive pro-otic cartilage and a slender post-palatine process are observed. These two cartilages do not meet and consequently the lateral commissure remains incomplete. In *Trichopodus* the lateral commissure appears in a 4·0 mm. larva as a thin slender plate-like structure forming the anterior boundary of the trigemino-facialis chamber. The pro-otic and the post-palatine processes are not found in the earlier forms examined.

The branches of the trigeminal as well the palatine branch of the facial run anterior to the lateral commissure. The remaining branches of the facial nerve lie within the trigeminofacialis chamber. The lateral commissure is ossified in a 6·0 mm. larva of *Trichopodus*.

In a 10·5 mm. larva of *Salmo* fario (de Beer, 1927) the cartilaginous rudiments of the auditory capsules are differentiated in the anterolateral corners of the otic-sacs and are connected with the anterior portion of the parachordals by a dense pro-cartilage. In *Gasterosteus* (Swinnerton, 1902) the auditory cartilage arises along with the parachordal and is never independent. Swinnerton (1902, p. 511) states that the stages during which the otic elements should be independent seem to be completely suppressed probably due to some influence at work hastening the development of that particular region. In *Trichopodus* the otic cartilage seems to appear slightly later than the parachordal as seen in stage one. Definite rudiments of the auditory cartilages appear a short time after and are connected with the parachordals by two commissures. This tends to show that the otic capsule is never independent though appearing slightly later than the parachordal. The fully formed auditory capsule generally shows external demarcation of the anterior, lateral and posterior semicircular canals in the teleosts. Internally the auditory capsule possesses one to three semicircular septa or partitions. In *Gobius* as stated by Norman (1926, p. 433) the lateral semicircular septum is chondrified, the posterior is fibrous and the anterior absent. Only two septa are seen in *Gasterosteus* (Swinnerton, 1902), *Syngnathus* (Kindred, 1921), *Cyclopterus* (Uhlmann, 1921), *Anguilla* (Norman, 1926), *Gambusia* (Ramaswami, 1945) and *Ophicephalus* (Srinivasachar, 1953). The anterior semicircular septum is absent.

In *Trichopodus* only two semicircular septa, namely the lateral and posterior, are present. The lateral septum which is generally vertical is a stout pillar-like cartilage, disposed sightly obliquely. The posterior septum is not very prominent.

The tectum synoticum unites the two auditory capsules dorsally in a number of teleosts like *Gasterosteus* (Swinnerton, 1902) *Cyclopterus*

(Uhlmann, 1921), *Anguilla* (Norman, 1926), *Salmo* (de Beer, 1927), *Gambusia* (Ramaswami, 1945) and *Ophicephalus* (Srinivasachar, 1953). In *Amiurus* (Kindred, 1919) the tectum synoticum is in continuation with the tectum posterius. In *Syngnathus* (Kindred, 1921) the tectum synoticum is very slender and is restricted only to the anterior region of the auditory capsules. A well developed tectum synoticum is present in *Trichopodus* and it extends posteriorly as a median conical process over the occipital region.

The auditory capsule is always connected with the anterior part of the parachordal by means of a cartilaginous bar, the anterior basicapsular commissure, situated behind the exit of the facial nerve in teleosts. Another commissure called the posteior basicapsular commissure is found between the exits of the glossopharyngeal and the vagus and it is present in *Amiurus* (Kindred, 1919), *Anguilla* (Norman, 1926) and *Salmo* (de Beer, 1927). A basicapsular fenestra is noticed between the two commissures. In these forms the exit of the vagus is through the jugular foramen and that of the glossopharyngeal is associated with basicapsular fenestra. In *Clupea* (Wells, 1922), *Gasterosteus* (Swinnerton, 1902), *Gambusia* (Ramaswami, 1945) and *Ophicephalus* (Srinivasachar, 1953) the glossopharyngeal and the vagus leave the cranium through a common aperture the metotic foramen, separated from the basicapsular fenestra by a basivestibular commissure. This is also true in the case of *Trichopodus*.

In *Clupea* (Wells, 1922) and *Sebastes* (Mackintosh, 1923) the mandibular arch arising originally as a continuous cartilaginous or pro-cartilaginous element, differentiates subsequently into the quadrate and Meckel's cartilage. Norman (1926) who later examined these two fishes states that a distinct suture is observed between Meckel's cartilage and quadrate. In *Gasterosteus* (Swinnerton, 1902), *Lepidosteus* (Veit, 1911), *Amia* (Pehrson, 1922), *Anguilla* (Norman, 1926) and *Salmo* (de Beer, 1927) the two elements of the mandibular arch arise as independent chondrifications.

In *Ophicephalus* (Srinivasachar, 1953) the pterygoid process of the pterygoquadrate bar appears as an independent piece of cartilage in 4·5 mm. larva and is situated on either side of the middle region of the ethmoid plate. It gradually extends posteriorly and in 6 mm. larva fuses with the quadrate. In the 24 mm. larva it again becomes discontinuous with the quadrate.

In *Trichopodus* the pterygoid process appears as an anterior extension of the quadrate and extends up to the ethmoid plate in a 4 mm. larva (stage 8). In a fully formed chondrocranium the pterygoid process is continuous with the quadrate and extends dorsal to the ethmoid plate.

The different elements of the hyoid arch chondrify independently in *Gasterosteus* (Swinnerton, 1902), and *Salmo* (de Beer, 1927). In *Lepidosteus* (Veit, 1911) and *Amia* (Pehrson, 1922) the symplectic arises as an independent chondrification in a continuous procartilaginous tract. In *Clupea* (Wells, 1922) and *Ophicephalus* (Srinivasachar, 1953) the quadrate fuses with the hyomandibular and remains continuous with it. In *Clupea* (Norman, 1926) the quadrate becomes independent of the hyosymplectic in a 30 mm. larva but in *Ophicephalus* (Srinivasachar, 1953) it is continuous with the hyomandibular even in the fully formed chondrocranium.

In *Trichopodus* the hyosymplectic appears as a continuous rod of cartilage in a 2·5 mm. larva (stage 2). The symplectic cartilage is at no stage free from the hyomandibular and hence, a hyosymplectic is present in the fully formed chondrocranium. The hyosymplectic ventrally touches the quadrate part of the palatoquadrate cartilage but does not fuse with it at any stage. The ceratohyal is also present but not directly connected with the hyosymplectic. According to Norman (1926) the interhyal (stylohyal) may be regarded as a new structure developed in correlation with the need for a stronger suspensorium for the remainder of the hyoid arch after the hyomandibular has moved forward. In *Lepidosteus* (Veit, 1911) and *Amia* (Pehrson, 1922) it develops as a separate cartilage in the ligament connecting the hyomandibular with the ceratohyal. The stylohyal is segmented off from the upper portion of the ceratohyal in *Salmo* (de Beer, 1927). In *Trichopodus* a procartilaginous stylohyal is noticed in a 2·8 mm. larva (Stage 2) and it is chondrified in the next stage.

The basihyal is chondrified independently of the copula in *Gasterosteus* (Swinnerton, 1902) but is connected with it by pro-cartilage. In *Trichopodus* the basihyal does not arise as an independent chondrification but appears to be continuous with the first copula. Even in the fully formed chondrocranium it is continuous with the copula and expands into a stout rod-like cartilage anteriorly.

In *Ophicephalus* (Srinivasachar, 1953) there is an elongated common copula which gives attachment to all the five pairs of branchial arches. In *Gambusia* (Ramaswami, 1945) and many other teleosts generally there are noticed two copulæ giving attachment to the different branchial arches. In *Trichopodus* two copulæ are noticed. The branchial arches develop mostly in the same manner as in *Salmo* (de Beer, 1927) and *Ophicephalus* (Srinivasachar, 1953).

SUMMARY

Eighty stages were collected for the study of the development of the chondrocranium of *T. trichopterus* and ten of them have been studied in detail.

(*a*) The notochord is noticed in stage 1 (2·4 mm.).

(*b*) The trabeculæ and the parachordals make their appearance as procartilages in stage 1 (2·4 mm.).

(*c*) In stage 2 (2·5 mm.) the ethmoid plate is developed. The trabeculæ unite posteriorly with the parachordals enclosing a wide hypophysial fenestra. The anterior basicapsular and the basivestibular commissures are formed. Rudiments of the occipital arch elements are seen. Meckel's cartilage and quadrate part of pterygoquadrate cartilage are developed but they are not united with each other. Two basibranchials are seen and the anterior one unites with the basihyal.

(*d*) In stage 3 (2·8 mm.) a small procartilaginous stylohyal appears.

(*e*) In stage 4 (3 mm.) the stylohyal becomes cartilaginous and connects the ceratohyal with the hyomandibular. Two pharyngobranchials are noticed.

(*f*) In stage 7 (3·5 mm.) the orbital and epiphysial cartilages make their appearance. The orbital cartilage is placed between the ethmoid and auditory regions but is not connected to them in this stage. The first three pharyngobranchials are formed.

(*g*) In stage 8 (4 mm.) the trabecula communis is developed. The median nasal septum and the lamina orbitonasalis are appearing. The orbital cartilage unites with the auditory capsule. The lateral commissure is developed. The metotic foramen is formed. The pterygoid process extends anteriorly up to the ethmoid plate.

(*h*) In stage 9 (6 mm.) the nasal septum has united with the sphenoseptal commissure of the orbital cartilage. The trabeculæ become discontinuous with the parachordals; hence the boundary of the hypophysial fenestra is obliterated. The auditory capsule which is well developed shows internally two septa. A synotic tectum is formed. The first epibranchial is formed and the third and fourth pharyngobranchials are united.

(*i*) In the fully formed tropitrabic chondrocranium (stage 10; 11·5 mm.) the two sphenoseptal commissures unite in the middle, forming a roof for the brain. The tænia tecti medialis and tænia tecti medialis anterior are developed. A membranous inter-orbital septum is present. The oblique

eye-muscles are attached to the trabecula communis. The pterygoid process extends dorsal to the ethmoid plate. Meckel's cartilage has a retro-articular process. The basihyal is still continuous with the first basibranchial.

REFERENCES

Agar, W. E. .. "The development of the skull and visceral arches in *Lepidosiren* and *Protopterus*," *Trans. roy. Soc. Edin.*, 1906, **45**, 49–64.

Assheton, R. .. "Development of skull in *Gymnarchus*, *Budgett Memorial Volume*, 1907, No. **14**, 293–420.

de Beer, G. R. .. "The early development of the chondrocranium of *Salmo fario*," *Quart. J. micr. Sci.*, 1927, **71**, 259–312.

——— .. *The Development of the Vertebrate Skull*, Oxford, 1937.

Berril, N. J. .. "The development of the skull in the Sole and the Plaice," *Quart. J. micr. Sci.*, 1925, **69**, 217–44.

Kindred, J. E. .. "The skull of *Amiurus*," *Illinois biol. Mongr.*, 1919, **5** (1), 7–120.

——— .. "The chondrocranium of *Syngnathus fuscus*," *J. Morph.*, 1921, **35**, 425–56.

Mackintosh, N. A. .. "The chondrocranium of the Teleostean fish *Sebastes marinus*," *Proc. zool. Soc., Lond.*, 1923, 501–13.

Moy-Thomas, J. A. .. "Notes on the development of the chondrocranium of *Polypterus senegalus*," *Quart. J. micr.Sci.*, 1933, **76**, 209–29.

Norman, J. R. .. "The development of the Chondrocranium of the Eel (*Anguilla vulgaris*), with observations on the comparative morphology and development of the chondrocranium in bony fishes," *Phil. Trans.*, 1926, **214 B**, 369–464.

Pehrson, T. .. "Some points in the development of Teleostomian fishes," *Acta Zool. Stockh.*, 1922, **3**, 1–63.

Ramaswami, L. S. .. "The chondrocranium of *Gambusia* (Cyprinodontes) with an account of the osteocranium of the adult," *J. Mysore Univ.*, 1945, **6**, 19–45.

Sewertzoff, A. N. .. "The development of chondrocranium in *Acipenser*," *Acta Zool. Stockh.*, 1928, **9** (cited by de Beer, 1937).

Srinivasachar, H. R. .. "The development of the chondrocranium in *Ophicephalus*," *Journ. Linn. Soc. Zool.*, 1953, **42**, 238–59.

Swinnerton, H. H. .. "A contribution to the morphology of the Teleostean Head Skeleton, based upon a study of the developing skull of the three-spined stickle back (*Gasterosteus aculeatus*)," *Quart. J. micr. Sci.*, 1902, **45**, 503–93.

Uhlmann, E. .. "Studien Zur Kenntnis des Schädels von *Cyclopterus lumpus* L. I. Morphogenese des Schädels," *Jena. Z. Natur.*, 1921, **57**, 275–314.

Veit, O. .. "Beitrage Zur Kenntnis des Kopfes der Wirbeltere; die entwicklung des Primordial-craniums von *Lepidosteus osseus*," *Anat. Hefte.*, 1911, **33**, 155–203 (cited by Norman, 1926).

Weber, M. and Beaufort, L. F. .. *The Fishes of the Indo-Australian Archipelago*, 1929, **4**, 366–67.

Wells, F. R. .. "On the morphology of the chondrocranium of the larval herring (*Clupea harengus*)," *Proc. zool. Soc. Lond.*, 1922, 1213–29.

van Wijhe, J. W. .. "A new method for demonstrating cartilaginous microskeletons," *Verh. Akad. Wet. Amst.*, 1902, **5**, 47.

Key to Abbreviations

abc .. anterior basicapsular commissure
ac .. auditory capsule
aep .. anterior ethmoid process
arm .. anterior rectus muscle
arp .. hiarticular process
ba 1–5 brancal alrches 1–5
bcf .. basicapsuar fenestra
bh .. basihyal
bp .. basal plate
br .. brain
bvc .. basivestibular commissure
cb 1–5 ceratobranchials 1–5
ch .. ceratohyals
cop 1–2 copulæ 1–2 or basibranchials 1–2
ct .. cartilage
ctr .. cartilaginous rod
eb 1–4 epibranchials 1–4
emc .. anterior eye-muscle canal
eof .. foramen olfactorium evehens
ep .. ethmoid plate
epb .. epiphysial bar
fh .. foramen in the hyosymplectic for the hyomandibularis facialis nerve
fme .. metotic foramen
hb 1–3 hypobranchials 1–3
hh .. hypohyal
hs .. hyosymplectic
hyf .. facet for the articulation of the hyosymplectic
iom .. inferior oblique muscle
irm .. inferior rectus muscle
lcm .. lateral commissure
lon .. lamina orbitonasalis
mc .. Meckel's cartilage
mcu .. Rami of Meckel's cartilage united anteriorly
n .. notochord
ns .. nasal septum
oa .. occipital arch
orc .. orbital cartilage
pc .. parachordal
phb 1–4 pharyngobranchials 1–4
pm .. posterior myodome
ppsc .. prominence of the posterior semicircular canal
ptp .. pterygoid process
ptq .. pterygo quadrate
ptsy .. posterior extension of tectum synoticum
qu .. quadrate
ra .. retroarticular process of Meckel's cartilage
rc .. rostral cartilage
rm .. rectus muscle
sh .. stylohyal
som .. superior oblique muscle
sphc .. sphenoseptal commissure
ssl .. septum semicircularis lateralis
ssp .. septum semicircularis posterius
tc .. trabecula
tcom .. trabecula communis
tfc .. trigeminofacialis chamber
tsy .. tectum synoticum
ttm .. tænia tecti medialis
ttma .. tænia tecti medialis anterior

SOME NEW SPECIES OF INDIAN HYMENOPTERA

By B. R. Subba Rao, B.Sc. (Hons.), Assoc.I.A.R.I., Ph.D., F.A.Z.
(*Division of Entomology, Indian Agricultural Research Institute, New Delhi*)

Received July 8, 1957
(Communicated by Dr. E. S. Narayanan, F.A.Sc.)

Introduction

The material dealt with in this paper was mainly collected by me and my colleagues. This collection was made under a Scheme for Survey of Parasites and Predators of Agricultural Crop Pests in the Indian Union.

Several of the genera, such as *Dusmetia* Mercet; *Chrysophagus* Ashmead; *Ectromatopsis* Compere; *Microterys* Thompson of the family Encyrtidæ are being recorded for the first time from India.

The types of the new species are deposited in the National Pusa Collections of the Division of Entomology, Indian Agricultural Research Institute, New Delhi.

Family EUPELMIDÆ

Solindenia amara new species

Since Cameron (1883) erected the genus *Solindenia* with *Solindenia picticornis* as genotype only one species has been recorded in India and described by Bhatnagar (1951).

While studying the parasite complex of *Hymenia recurvalis* L. this Eupelmid was reared as a hyperparasite of *Apanteles delhiensis* new species (description of the new species is under publication). In all, four females and one male were reared in the laboratory.

Female

Length.—3·3 mm. Head metallic with blue green iridescence and a coppery lustre at certain angles; viewed from above clothed with silvery white pubescence, rugulosely closely punctate. Ocelli large, arranged in an accute triangle; interocellar space slightly more than ocellocular space, front ocellar space slightly more than the interocellar space. Occiput concave. Antennæ inserted just above the mouth border and widely separated. Genæ and face below the middle covered with long closely set setæ. Face with a shallow scrobe and the front ocellus placed outside the furrow. Head from side slightly more than half as wide as high; post-orbital space closely punctate. Antennæ dark brown, metallic, slender. Scape lemon yellow,

slender, slightly more than half of flagellum. Pedicel brilliant metallic green, about one-fifth of the length of scape. Ring joint broader than longer. Funicle dark brown, segments one to three subequal, fourth segment shorter than third, fifth and sixth shorter than fourth, seventh longer than sixth. Club slightly less than half of the scape, apical joint longest. Eyes lemon yellow.

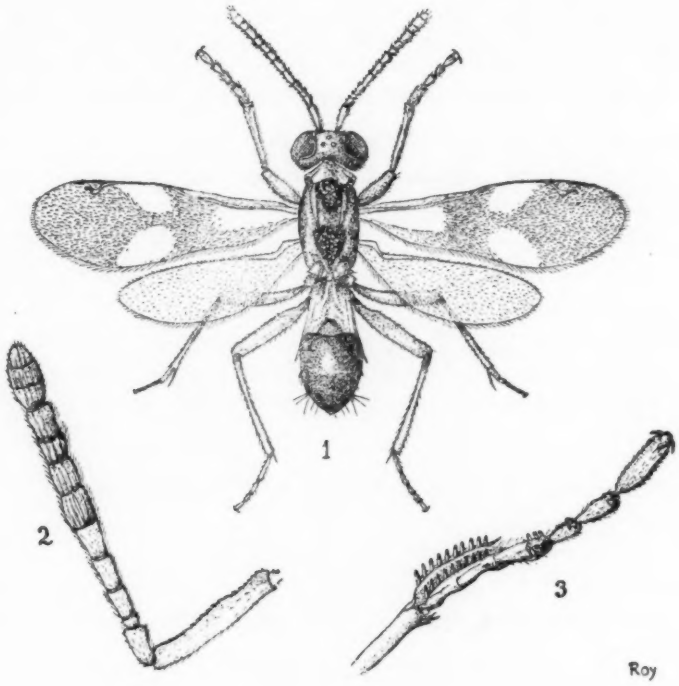


FIG. 1.—1. *Solindenia amara* new species, female. 2. Antenna of female. 3. Mid-tarsus of female.

Thorax.—Prothorax very narrow, narrower than head. Parapsidal furrows incomplete. Scutum slightly raised, coppery metallic, rugulosely closely punctate. Tigulæ slightly raised, dark brown. Scutellum metallic, rugulosely punctate. Forewings fuscous, with hyaline areas as shown in the figure. Forewing about two and half times as long as wide, marginal fringe moderate. Discal ciliation thick. Marginal vein slightly longer than the sub-marginal. Post-marginal vein twice longer than the stigmal. Legs with coxæ dark brown. Hind tibia not compressed, dark brown except

at apical one-third which is whitish; hind tarsus pale yellow. Hind tibial spur one-third the basi tarsus, armed with dark short denticles.

Abdomen slightly shorter than the head and the thorax put together, finely rugose punctate, first segment pale yellow, rest dark brown. Ovipositor concealed.

Holotype.—One female on celluloid strip, labelled hyperparasite on *Apanteles delhiensis* new species collected M. Ramachandra Rao. Type locality New Delhi. Paratypes: 3 females on celluloid strips.

KEY TO INDIAN SPECIES OF *Solindenia* CAMERON

1. Pedicel brilliant metallic green. Eyes pale yellow. First segment of abdomen pale yellow, rest dark brown. Ovipositor concealed .. .. *Solindenia amara Sp. nov*

2. Antennæ uniformly dark brown. Abdomen dark, wings hyaline with an oblique hairless line in the middle. Ovipositor exerted *Solindenia vermai* Bhatnagar.

Male

Essentially similar to the female except for its smaller size.

Type locality.—Delhi.

Host.—Apanteles delhiensis new species.

Collected by M. Ramachandra Rao, August, 1956.

Family APHELINIDÆ

Perissopterus cheriani Mani (*Ind. J. Ent.*, 1939, **1**, 88)

Marietta Motschulsky, 1863

Marietta cheriani (Mani)

I refer to this species as *Marietta cheriani* (Mani). Mani (1939) described this species from two females collected by Cherian. I have bred this species in large numbers on *Aspidiotus glomeratus*, a periodic pest on sugarcane in Delhi. The description of Mani is inadequate and I have based this description after examining a series of specimens. In addition to this the description of the male is also given.

Female

Length.—1·4 mm. Head wider than thorax, normal, vertex narrow but the frons are not at right angles to the vertex as shown in the figure by Mani. The holotype of Mani resembles the figure. But in natural condition and also when properly set the specimens do not shrink so much as seen in case of the holotype. Lateral ocelli close to the eye border. Inter-ocellar space twice more than the ocellocular space. Inner orbital borders converge downwards to form a triangle. Eyes grey. Antennæ six segmented. Scape twice longer than the pedicel. Funicular segments very narrow. Four strong reddish brown spurs just above the insertion of the antennæ.

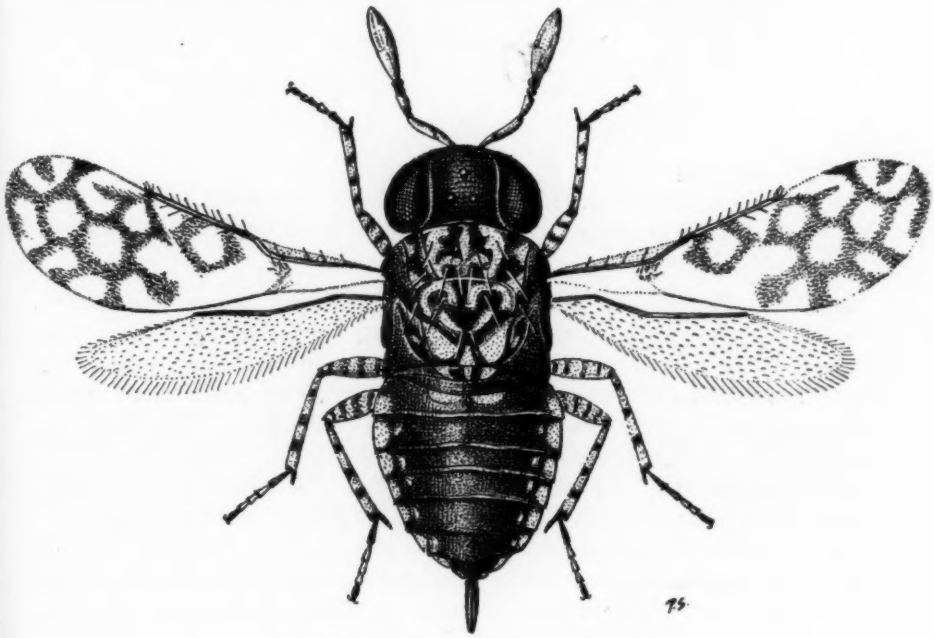


FIG. 2. *Marietta cheriani* (Mani), female.

Thorax.—Yellowish grey. Scutellum with or without two large subquadrate reddish brown spots in the middle. Generally only two spines on the posterior margins of the scutum and scutellum present. However, four spines on the scutellum is not uncommon. More spines are present on and lateral regions of the thorax as shown in the figure. Hind wings narrow. Transverse black stripes on the hind tibiæ relatively more widely

spaced, the posterior one being subapical. Fifth and the sixth tergites wit out conspicuous markings.

Male

Length.—1·07 mm. Essentially similar to the female. Reddish brown spot on the scutellum absent. The two spines on the posterior margins of the scutellum and scutum present. But the other spines are fewer. Thorax more reticulately punctate.

Allotype.—One specimen on card strip. Several paratypes on slides.

Host.—Aspidiotus glomeratus Green.

Type locality.—Delhi. Collected by Rana Brinder Kaur.

Family ENCYRTIDÆ

Subfamily ENCYRTINÆ

Anabrolepis mayurai new species

Female

Length.—1·25–1·75 mm. Brilliant metallic blue green with red iridescence on the thorax and vertex. Abdomen dark, shining. Eyes, brick red. Legs black, shining.

Head.—Normal, as broad as thorax. Frons and vertex very narrow, closely finely punctate; bare. Ocelli reddish brown, arranged in an acute angle. Ocellocular space very narrow, lateral ocelli placed very close to the orbits. Front ocellar space more than inter-ocellar space. Inter-orbital space slightly less than one-third of the width of the head. Eyes brick red, microscopically thinly pubescent. Genæ prominent. Seen from front the lower borders of the orbit are not parallel. Antennæ inserted slightly below the middle of the face, just above an imaginary line joining the lower orbital borders. Antennal fovæ short but semicircular. Antennæ yellowish brown, eleven-segmented. Scape pale brown, slightly dilated, more than thrice the length of the pedicel. Funicle six-segmented, broader than long, but the segments increase in length from base to apex. Club three-segmented, swollen, more or less spindle-shaped, longer than the funicle.

Thorax.—Entire thorax brilliant metallic bluish green. Mesonotum leathery punctate, short silvery white pubescence present. Scutellum closely reticulately punctate. Metanotum and propodeum black, shining, shagreened. Axillæ connate.

Legs.—Coxæ black. Trochanter pale yellow. Femur of fore and hind legs dark brown with bases pale yellow. Tibia of fore and hind legs dark

brown with their apices pale yellow. Femur of mid-legs pale yellow, tibia yellowish white. Tarsus pale yellow.

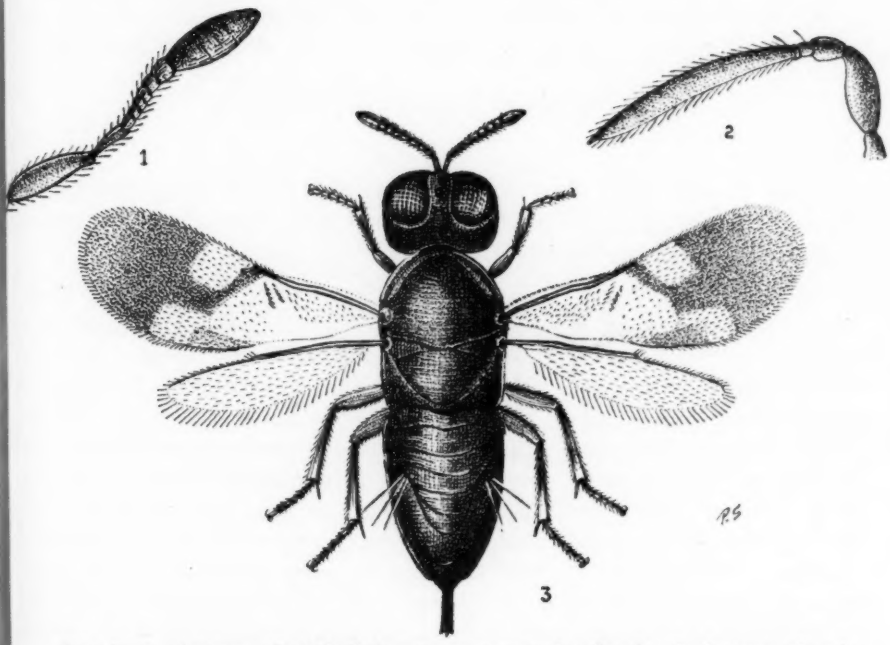


FIG. 3.—1. Antenna of *Anabrolepis mayurai* new species, female. 2. Antenna of male. 3. *Anabrolepis mayurai* new species, female.

Wings.—Fuscous with several rays along the margin alternating with hyaline spots. Marginal vein punctiform, longer than broad. Stigmal vein longer than post-marginal vein. Fore wings slightly more than twice longer than wide.

Abdomen dark brown to black, shiny, concave. Ovipositor exerted.

Male

Essentially same as female with the exception of the following characters:

Head and thorax dull metallic green with red iridescence. Slightly smaller than the female. However, there is a great variation in the size among males. Antennæ five-segmented. Scape, pedicel, two jointed funicle and a long undivided club. Scape two and half times the pedicel. Club five and a half times the pedicel. Funicular segments broader than long. Forewing twice longer than broad. Wings hyaline with an oblique hairless line. Abdomen dark brown, non-metallic.

Holo- and allo-types on card mount, collected by Rana Brinder Kaur, February 1957. This is the first record of the genus from India.

Type locality.—New Delhi.

Host.—Aspidiotus glomeratus Green.

Chrysopophagus bangalorensis new species

Female

Length.—1·6 mm. Brown colour in general, with dark patches on the thorax and posterior part of the abdomen. Eyes black to dark brown. Scape and pedicel brown, funicle black, club pale brown.

Head viewed from above lenticular. Vertex narrow. Viewed from front longer than wide, finely reticulately punctate. Ocelli in an equilateral triangle. Inter-ocellar space twice more than ocellocular space. Face in lateral aspect sub-triangle. Eyes reach downward about three-fourth way. Scrobes very short, shallow, twice as long as the antennal sockets. Genal suture faintly marked below the eyes. Antennæ inserted close to the oral margin, the sockets about three times their own diameter apart. Antennæ long, slender, slightly dorsoventrally flattened. The scape reaching only midway through the length of the head, slender, yellowish brown. Pedicel smaller, yellowish brown. The funicular segments flattened, black, first segment longer than broad, second segment wider than long, third and fourth segments almost as long as wide, fifth and sixth wider than long. Club three-segmented, first segment long, black, second segment dark only in the posterior region, the third being yellowish. Apical segment of the club rounded at apex. Antennal segments measure as shown below.

Scape, 3·75; pedicel, 1·2; funicle, 4·3; club, 3.

Thorax.—Thorax of normal Encyrtid type. Mesoscutum strongly convex, dark, broader than long, closely coarsely punctate, hairy. Axillæ connate. Scutellum convex, lighter in colour, longer than scutum. Apex of scutellum with a tuft of strong black bristle-like hairs. Wings fuscous, more than three times longer than width. Sub-marginal two and a half times longer than the marginal, thickened at base. Marginal vein slightly more than seven times longer than its own breadth. Post-marginal vein short, thick. Stigmal vein slightly longer than the marginal. Marginal fringes moderately short.

Legs.—Coxæ of all legs lemon yellow, rest of the legs deep yellow. Middle femur and tibia with dark brown patches.

Abdomen.—Conical, slightly shorter than the head and thorax combined. In card mounts dorsum of the abdomen depressed, but in life the abdomen is as long as the head and thorax combined and not depressed. First segment dark brown, second segment light yellow and the rest dark brown. Ovipositor slightly exerted.

FIG. 4. *Chrysopophagus bangalorensis* new species, female.

Sculpture.—Axillæ and scutellum with fine close timble-like punctures formed by reticulated lines. Mesoscutum broadly punctate. Frontovertex minutely punctate.

Male

Length.—1·4 mm. Head much thinner fronto-occipitally than in female. Tuft of hair on the scutellum absent. Abdomen stout, shorter than the head and thorax combined, ovate. First and second segments pale yellow and the rest dark brown. Antennæ dark brown throughout. Wings hyaline.

Holo- and allotypes on card strips. This is the first record of the genus from India.

Host.—Antonina indica Green (Rodes grass scale).

Type locality.—Bangalore. Collected by H. S. Sangwan, 3-4-1957.

Ectromatopsis indica new species

Female

General colour bright yellow with dark patches. Abdomen conic ovate, very much narrower than thorax. Eyes black. Scutellum with a few black spines. Length 1·25 mm.

Head.—Frontovertex longer than broad. Ocelli small, ocellocular space twice inter-ocellar space. Front ocellar space narrower than ocellocular space. Ocelli arranged in equilateral triangle. Vertex smooth, shining. Antennæ brown, scape seven times longer than its maximum breadth, yellow. Pedicel short, one-third of the scape, twice longer than its own breadth, brown. Funicle six-segmented, slightly smaller than the scape, second funicular segment shortest. Club three-segmented, slightly smaller than the funicle. Antennæ inserted slightly above the oral border, the sockets about twice their own diameter apart. Scrobes slightly more than twice longer than the antennal sockets.

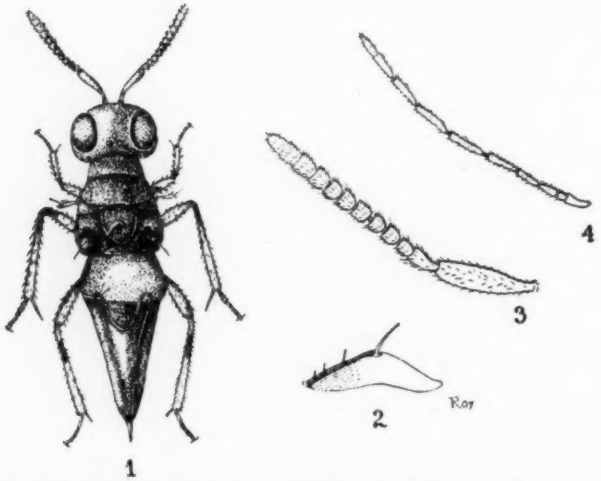


FIG. 5.—1. *Ectromatopsis indica* new species., female. 2. Fore wing of female. 3. Antenna of female. 4. Antenna of male.

Thorax.—Brownish. Prothorax narrow. Propodeum very much broadened. Scutum with a number of hard bristle-like hairs. Scutellum yellow. Apex of scutellum with seven black strong bristles.

Wings.—Fore wings very much reduced. Only a trace is seen in prepared slides. Sub-marginal very strong.

Abdomen conically produced posteriorly. Ovipositor slightly exerted. Fore- and mid-legs yellow. Hind legs yellow with dark patches. Mid-tibial spur longer than mid-metatarsus.

Male

Essentially similar to the female, but slightly smaller. Wings hyaline. Length 0·8 mm. Antennæ uniformly light yellow.

Holo- and allotypes on card strips. This is the first record of the genus from India.

Type locality.—Bangalore. Coll. H. S. Sangwan, 3-4-1957.

Host.—Antonina indica Green.

Family ENCYRTIDÆ

Subfamily ENCYRTINÆ

Dusmetia sangwani new species

Female

Apterous, dark brown, robust. Antennæ, scape and pedicel yellow, funicle dark brown, club white. Eyes grey. Length 1·3 mm. Head viewed from above lenticular, viewed from front, much broader than long. Vertex broad. Eyes small. Ocelli arranged in an equilateral triangle. Inter-ocellar space very slightly more than the ocellocular space. Vertex and frontovertex punctate, punctures distributed sparsely. Eyes reach downward about half-

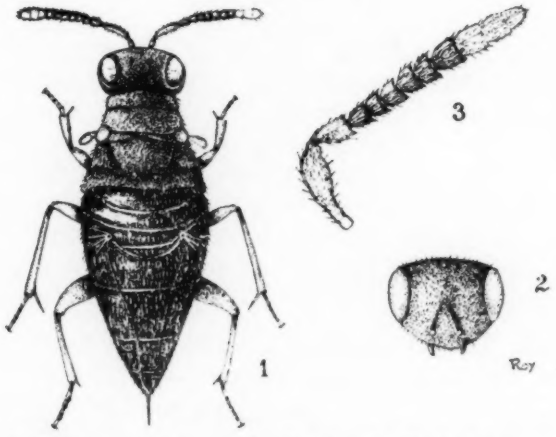


FIG. 6.—1. *Dusmetia sangwani* new species, female. 2. Head of female. 3. Antenna of male.

way. Scrobes very shallow. Face in lateral aspect almost an equilateral triangle. Genal suture very faintly marked below the eyes. Antennæ inserted very close to the oral margin, the sockets about two times their own diameter apart. Scape yellow, two and a half times longer than pedicel. Pedicel one-third of funicle, which is dark brown, six-segmented. First funicle segment longer than broad, second broader than long, the rest almost equal. Club three-segmented, twice the length of pedicel, apical segment rounded, white, opaque.

Thorax.—Slightly narrower than the head. Prothorax broader than mesoscutum, which is twice broader than long. Axillæ very narrow. Scutellum normal. Entire thorax brown, beset with regularly arranged dark bristle-like hairs. Wings very much reduced, only a trace of the fore wing is seen.

Legs.—All legs yellow. Forelegs short and stout. Mid-tibial spur a shade smaller than the mid-metatarsus.

Abdomen.—Dark brown, broader than the thorax, longer than the head and thorax combined, depressed slightly in dry specimens but not so in live specimens. Hypopygium is well developed and prominent. Ovipositor slightly exerted.

Male

Length 1 mm. Black, winged, wings hyaline. Fore legs brown, rest yellow.

Head viewed from above, vertex broad, coarsely punctate. Ocelli large, brown. Inter-ocellar space twice the ocellocular space. Eyes very small, black. Head viewed from front broader than long. Antennæ inserted close to the oral margin. Scape yellow, much dilated. Pedicel less than half of scape. Pedicel dorsoventrally curved slightly to form a sort of arch. Funicle six-segmented, the first being the largest. Club three-segmented, rather short. Fore wings hyaline. Marginal vein punctiform, broader than long, eight times smaller than the sub-marginal vein. Stigmal vein short. Abdomen dark, much shorter than the head and thorax combined.

Holo- and allotypes on card strips. This is the first record of the genus from India. Several paratypes on slides.

Type locality.—Bangalore. Collected by H. S. Sangwan, 3-4-1957.

Host.—*Antonina indica* Green.

Microterys delhiensis new species

Female

Head in dorsal view wider than long, frontovertex longer than wide and slightly more than one-third as wide as the head. Ocelli in an equilateral triangle or nearly so, the posterior pair slightly less than their own diameter from the orbital borders but at least four times away from the occipital margins. Head frontal view a shade wider than high. Antennal sockets located about their own length above the oral margin and about twice this distance from the base of the eyes. Scrobes well impressed, triangular, meeting above. Elevated portion between the scrobes rounded out. Eyes extended downward about half-way, their length slightly more than that of cheeks. Mandible broad with three distinct teeth, the apical being the longest.

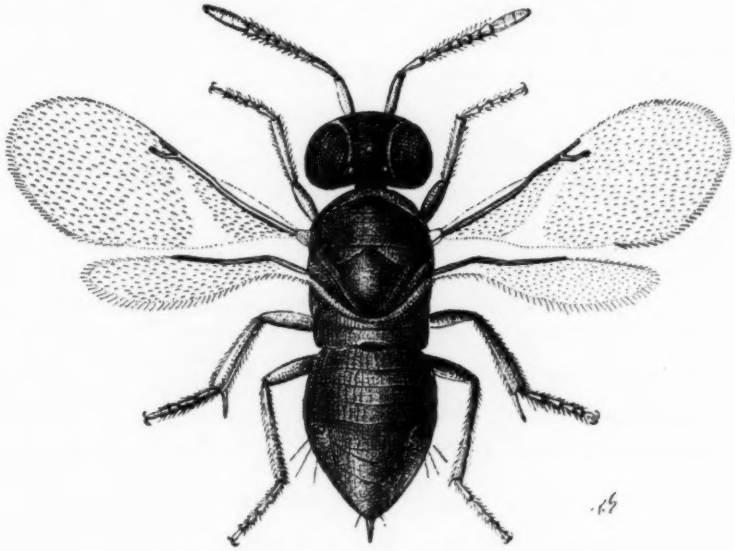


FIG. 7. *Microterys delhiensis* new species, female.

Antennæ.—Scape very slighly expanded, slightly compressed only towards the apex, yellow. Pedicel slightly longer and wider than the first funicle joint, first funicle joint is the longest and the rest slightly smaller, but equal in size. However, the segments become broader progressively. Club oblong, three times longer than broad.

Thorax.—Stongly convex from side to side. Mesoscutum about as long as scutellum. Axillæ connate.

Abdomen.—Sub-triangular or more towards conic-ovate, longer than wide, shorter than head and thorax combined. In dried specimens, tergum strongly depressed. Paratergites clear only in prepared slides. Ovipositor slightly exerted.

Wings.—Fore wings hyaline. Marginal vein at least three times longer than thick. Post-marginal nearly equal to marginal but subequal to stigmal.

Sculpture.—Frontovertex reticulate punctate, face and cheek also reticulate punctate. Genal suture distinct. Mesoscutum and tegulæ reticulate. Scutellum with sculpture slightly different from that of scutellum. In well prepared slides the reticulated lines more zig-zag.

Colour.—Face and cheeks pale yellow. Mandible dark brown. Frontovertex brown, mesoscutum light brown. Sides of propodeum dark. Abdominal tergites dark brown. Basal tergites at corners brilliant metallic bluish green. All legs pale yellow.

Length 2·1 mm. Wing expanse 3·5 mm.

Male

Length.—1·75 mm. Essentially similar to the female except for the following characters:

Face and frontovertex closely reticulately punctate, metallic bluish green with slight red iridescence on the vertex only. Antennæ pale yellow, cylindrical. Prothorax brown. Axillæ yellow, connate. Scutellum metallic green, propodeum yellow. Abdomen blackish brown.

Holo- and allotypes on card strips.

Host.—Saccharicoccus sacchari (Ckll.)

Type locality.—New Delhi. Collected by Rana Brinder Kaur.

Family THYSANIDÆ

Thysanus ranæ new species

Female

Black, shining, robust. Marginal fringes long. Head crescentic, some what variable in proportion, acutely margined behind. Eyes scarcely but not reaching the hind margin. Ocelli in an obtuse triangle. Inter-ocellar space twice ocellocular space. Head in frontal view with malar space seven-ninths the longer diameter of eye. Scrobes shallow and broader than high, triangular. Head smooth. Vertex shining. Mandibles bidentate. Antennæ with scape weakly rounded below and slightly dilated above,

slightly more than seven times longer than the greatest breadth. Pedicel slightly less than half of scape in length and three times longer than its own breadth. Funicle half of pedicel, clearly four segmented, three times longer than the pedicel, five times longer than its own breadth, slightly shorter than the remainder of the antennæ.

Pronotum comparatively broad, mesonotum more than one and a half times the meso-scutellum. Axillæ distinctly separated in prepared mounts. Propodeum V-shaped, much elongated and pushed back over the abdominal tergites. Entire thorax shining and jet black. Fore wings fuscous. Marginal and submarginal almost equal, but marginal slightly smaller than submarginal. Marginal cilia of both wings more than half the width of the wing. Hind wings spoon-shaped, radius extending to the hooklets.

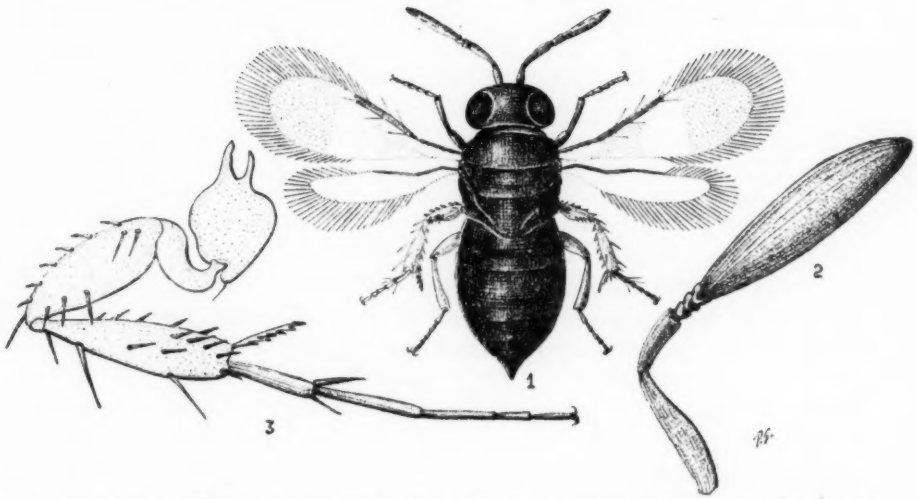


FIG. 8.—1. *Thysanus ranæ* new species, female. 2. Antenna of female. 3. Mid-leg of female.

Fore-legs short. Except tarsal segments which are testaceous the rest are dark brown. Mid-legs with femora strongly dilated below, greatest width is slightly shorter than half of the length, bearing stout hairs arranged as shown in the figure; the tibial spur also bears strong pectination. Tibial spur slightly less than the length of the basi tarsus. Abdomen oblong, the sides being almost parallel. Ovipositor not exerted, but strongly defined.

Male

Essentially similar to the female except for its shorter length. Holotype and allotype on card mounts. Several paratypes on slides.

Type locality.—New Delhi.

Host.—Probably a perparasite on an Encyrtid parasitising the sugarcane mealy-bug *Saccharicoccus sacchari*; collected by Rana Brinder Kaur.

The genus *Thysanus* was erected by Walker in 1840, with *Thysanus* as genotype. Later (1880) Ashmead erected the genus *Signiphora* with *Signiphora flaopalliatus* as the genotype, and placed it under the subfamily Encyrtinæ, which was accepted by Howard also. However, Schmiedeknecht, who classed the whole of Chalcids as single family, kept the Euplemids and Encyrtids as separate subfamilies, but gave the Signiphorids the status of a tribe within the group Encyrtinæ. Mercet (1917) in his work brought these two genera together in a single tribe, but Silvestri (1918) synonymised the genus *Signiphora* with *Thysanus*. It was A. B. Gahan, who actually treated *Signiphora* as a straight synonym of *Thysanus*. However, Peck (1951) elevated this group of insects to the status of a family equivalent to that of Eupelmidæ and Encyrtidæ. This classification has been accepted by Kerrich (1953) and he has given an excellent review on the systematic position of this peculiar family which consists of but a single genus. Though several genera were erected since Walker, they are all more or less now considered to be synonyms of the genus *Thysanus*.

ACKNOWLEDGMENT

I am grateful to Dr. E. S. Narayanan, Head of the Division of Entomology, for his encouragement and facilities. My thanks to Dr. Harold Compere for checking some of my identifications.

REFERENCES

1. Ashmead, W, H. .. *Orange Insects*, 1880, pp. 29–30.
2. Bhatnagar, S. P. .. *Ind. J. Agric. Sci.*, 1951, **21**, Part II.
3. Cameron, P. .. *Trans. Ent. Soc. Lond.*, 1883, p. 189.
4. Mani, M. S. .. *Rec. Ind. Mus.*, 1936, **37**, 131–32.
5. Mercet, R. G. .. *Rev. Acad. Madr.*, 1917, **16**, 160–70.
6. Peck, O. .. *Superfamily Chalcidoidea. Synoptic Catalogue, Agric. Monograph.*, U.S.D.A., No. 2., 1951.
7. Silverstri, F. .. *Boll. Lab. Zool. Portici*, 1918, **12**, 266–71.
8. Walker, F. .. *Ann. Nat. Hist.*, 1840, **4**, 234.
9. Kerrich, G. J. .. *Bull. Ent. Rev.*, 1953, **44**, 789–810.

STUDIES IN SAMPLING TECHNIQUE

VI.* Rind Hardness in Sugarcane

By S. L. Sharma, M.Sc.
(Sugarcane Mycologist, Central Sugarcane Research Station, Pusa, Bihar)
AND
Rajeshwar Prasad, B.Sc. (Ag.)
(Junior Botanical Assistant, Central Sugarcane Research Station, Pusa, Bihar)

Received April 13, 1957
(Communicated by Shri K. L. Khanna, F.A.Sc.)

I. Introduction

The rind hardness of a sugarcane variety is as important a character as its sugar content because it determines its usefulness in ways more than one. While the cane is growing in field, it protects it from the ravages of rodents such as rats and squirrels and of larger animals like jackals. Whereas red rot wiped out Co. 210 and Co. 213 from the scene, the jackals were instrumental, to some extent, in the reduction of area under Co. 313, a soft rinded variety, over major portion of the white sugar belt in this State. It has also been found to determine the resistance of a variety to stem borers. Buzacott (1940) and Rao (1941 and 1951) found rind hardness to be positively correlated with the resistance of a variety, respectively to *Rhabdocnemis obscura* Boisd and *Diatreæ venosata* Walkers. The latter author while reviewing literature on the subject cited several workers who found either fibre-content or rind hardness of a variety among the major factors in imparting resistance to stem borer. Khanna and Panje (1937) and Khanna and Sharma (1949) have shown the two to be interdependent on each other as they are the manifestation of one and the same phenomenon, namely, the lignification of tissues. Rind also gives protection to cane against fungus diseases, the causal organisms of which, therefore, have to enter a stalk through such weak spots in its armour as root-eyes, buds, or leaf scars.

The sum total of this protection, afforded by the rind against rodents, jackals, stem borers and fungus diseases, is reflected in the ultimate yield in which the cultivator is mainly interested.

---

* Parts I–V have appeared in *Proc. Ind. Acad. Sci.*, Sec. B, respectively in **28,** 107–30; **31,** 33–44; **31,** 111–19; **32,** 269–88; and **34,** 210–19. Abstract in *Proc. 39th Ind. Sci. Cong.*, 1952, Part III, 290.

As regards the role of rind, in other words, of fibre, in processing sugarcane into sugar, Khanna and Sharma (1949) have shown how the milling properties of certain varieties were determined both by its quantity and quality. The use of bagasse major bulk of which is formed by rind, for the manufacture of paper, has opened an entirely new avenue for this waste product hitherto used only as fuel either in boilers in a sugar factory or in domestic ovens in this country.

It would, therefore, appear that study of rind hardness was fairly important in assessing agronomical as well as milling characteristics of a variety. But there exists little unanimity among different workers (Khanna and Panje, 1939; Ueno, 1938; Buzacott, 1940; and Rao, 1941 and 1951) as to how it should be estimated. A detailed study for the accurate determination of this character was, therefore, undertaken so that a method might be evolved for use in the selection of varieties for different categories of canes such as chewing, gur and factory, from this point of view.

II. MATERIAL AND METHODS

The investigations reported in this contribution were carried out in two seasons (1949–50 and 1950–51) on the following varieties:

1949–50: Co. 453, B.O. 10, B.O. 11, B.O. 21 and B.O. 24.

1950–51: Co. 453, Co. 513 and B.O. 11.

Method A.—In the first season a cane stalk was taken as a unit, while in the second year it was a clump which formed the basis of its determination. Well developed, non-arrowing stalks, free of borers and fungus diseases, were selected, one each (the best developed one) from 100 clumps of a variety from a non-waterlogged field. Each cane was stripped up to the node bearing the standard leaf and divided into three portions on length basis, and all the eight measurements for this character were taken at half the distance from leaf scar to leaf scar, of the middle internodes of these three portions, four for the middle portion at the ends of the median and lateral diameters and two each for bottom and top portions at the ends of the lateral only.

Method B.—In 1950–51, clump being the unit, all the millable canes of 6 rows, 3 feet apart and 60 feet long, covering nearly 1/40th of an acre which was the usual size of an experimental plot for this crop, were tested for rind hardness after dividing the plot into 120 units each 3 feet $\times$ 3 feet. It was measured only on the side opposite the bud at the mid-point of the middle internode determined on length basis.

All measurements were taken with the rind hardness apparatus (Fig. 1) designed at the Station (Khanna, 1935).

III. Results

A. 1949–50

TABLE I

Average rind hardness of five varieties at different points*
(1949–50)

Region	Location†	Co. 453	B.O. 10	B.O. 11	B.O. 21	B.O. 24
Bottom ..	I	14·99±0·17	13·89±0·17	13·46±0·13	11·66±0·12	13·81±0·14
	III	14·78±0·15	13·97±0·15	13·57±0·13	11·51±0·13	13·74±0·20
Middle ..	I	13·59±0·16	12·80±0·11	12·86±0·12	10·97±0·14	13·39±0·14
	II	13·72±0·15	12·54±0·11	12·57±0·10	10·75±0·11	12·98±0·09
	III	13·17±0·15	12·91±0·13	12·66±0·11	11·01±0·12	12·87±0·13
	IV	13·35±0·14	12·73±0·10	12·61±0·08	10·86±0·11	13·07±0·13
Top ..	I	10·84±0·14	9·42±0·12	10·02±0·11	7·39±0·09	9·55±0·10
	III	11·04±0·16	9·62±0·12	10·41±0·11	7·47±0·09	9·61±0·10

* Average of 100 measurements.

† At the end of the median diameter on the bud side—I.
At the end of the lateral diameter on the right side—II.
At the end of the median diameter opposite the bud—III.
At the end of the lateral diameter on the left side—IV.

(1) *Sector.*—Average hardness of rind in different sectors, as given in Table I, was not found to be significantly different when compared with each other within the internode itself, except in the following five pairs as tested by the formula

$$\frac{m_1 - m_2}{\sqrt{S_1^2 + S_2^2}}\ \dagger$$

Co. 453: Middle internode; II and III at 1% level.
B.O. 10: Middle internode; II and III at 5% level.
B.O. 11: Top internode: I and III at 5% level.
B.O. 24: Middle internode; I and II at 5% level.
B.O. 24: Middle internode; I and III at 1% level.

† Where m_1 and m_2 are the two means and S_1 and S_2 their standard errors.

The average hardness of rind at the four points, therefore, was not materially different from one another, and any one of them might be taken to represent an internode.

(2) *Internode.*—In order to see how far a stalk, as whole, was represented by one of its components, namely, the internodes, correlation coefficients between the rind hardness of the former and that of the three internodes studied, were worked out separately after omitting the measurements done at II and IV for the middle internode and thus avoiding a bias in its favour. It was found that in three of the varieties studied, *viz.*, Co. 453, B.O. 11 and B.O. 24, the correlation coefficient was highest in the case of the middle internodes (Table II) while in that of the other two, the bottom internode was found to give a better picture of the stalk. This naturally raised the question as to whether it was really so, in other words, whether the correlation coefficients for bottom and middle internodes were significantly different from one another.

TABLE II

Correlation coefficients between hardness of a stalk and that of an internode

Varieties	r_{bs}	r_{ms}	r_{ts}	Value of t for the transformed r into z		
				Between 2 and 3	Between 2 and 4	Between 3 and 4
1	2	3	4			
Co. 453 ..	0·7808†	0·9196†	0·7923†	1·79	0·10	1·69
B.O. 10 ..	0·8590†	0·8206†	0·6179†	0·43	1·88	1·45
B.O. 11 ..	0·7219†	0·7414†	0·2949†	0·14	2·15*	2·02*
B.O. 21 ..	0·8439†	0·6329†	0·5619†	1·62	1·99	0·37
B.O. 24 ..	0·6992†	0·8335†	0·6737†	1·11	1·27	0·16

1. * denotes significance at 5% level. † denotes significance at 1% level.
2. b = Rind hardness of bottom internode.
   m = Rind hardness of middle internode.
   t = Rind hardness of top internode.
   s = Rind hardness of the stalk.

On applying t test after converting r into z by the formula:

$$z = \tfrac{1}{2} \log_e \frac{1+r}{1-r},$$

it was found that the two correletion coefficients both for B.O. 10 and B.O. 21 were not significantly different from each other. So for these two varieties also, middle internode could as well be taken.

(3) *Stalk.*—Statistical analysis of the data collected from 100 stalks showed that variation within the sample was of a very low order in all the five varieties because in no case did the standard error expressed as percentage of the mean, exceed 1·38 (Table III). Variance due to stalk was found to be 0·00% of the total variance of the sample for all of them.

B. 1950–51

Rind hardness for the three varieties measured during this season is given in Table IV along with the total variance in the sample and its distribution amongst the four hierarchies, namely row, unit, clump and stalk.

As was expected, the averages for both Co. 453 and B.O. 11 were less than those of the preceding season, and stalk was overwhelmingly responsible for the variation within the sample. Unit as a hierarchy was found to be more important than clump because whereas clumps had more or less the same number of stalks of approximately corresponding ages, units had different number of clumps ranging from nil to as many as three of them. Row was not of much consequence in the determination of this character.

IV. Discussion

A. Role of different hierarchies.—In the methods of sampling, variation was brought in by six hierarchies, namely, (i) sector, (ii) internode, (iii) stalk (common to both the methods), (iv) clump, (v) unit and (vi) row, depending upon whether sampling was done on the basis of selection of stalks or of entire population within a specified area. It was, however, not possible to include all the six hierarchies in one sample for obvious reasons.

In 1949–50 rind hardness of different sectors of the same internode was not found to be significantly different from one another (Table I) nor was the variance due to this hierarchy greater than 18% of the total variance in four out of the five varieties. Only in the case of Co. 453, it was found to be 23·53% (Table III). Thus sector as a hierarchy was not found to be of much importance. Khanna *et al.* (1950) who studied, in detail, the first three hierarchies for sampling technique for anatomical features, came to a similar conclusion so far as the area under vascular bundles per unit area was concerned. They found that

TABLE III

Estimated variance due to different zones in the rind hardness of five sugarcane varieties (1949–50)

Varieties	Mean	S.E.	S.E. as % of mean	σ_1^2	σ_1^2 expressed as % of total variance	σ_2^2	σ_2^2 expressed as % of total variance	σ_3^2	σ_3^2 expressed as % of total variance
Co. 453 ..	13·16	0·1270	0·97	0·00	0·00	4·1934	76·47	1·2900	23·53
B.O. 10 ..	12·06	0·1432	1·19	0·00	0·00	5·7259	87·03	0·8533	12·97
B.O. 11 ..	12·18	0·1090	0·89	0·00	0·00	3·2192	82·42	0·6867	17·58
B.O. 21 ..	10·01	0·1382	1·38	0·00	0·00	5·2909	85·74	0·8800	17·02
B.O. 24 ..	12.18	0·1362	1·12	0·00	0·00	5·1184	85·05	0·9000	14·95

1. Where σ_1^2 = variance due to stalks; σ_2^2 = variance due to internodes; σ_3^2 = variance due to sectors.

2. If the mean square due to a particular hierarchy happens to be less than that in the immediately next one, the variation due to that hierarchy has been taken to be zero.

3. Mean for a variety was calculated from 600 observations; 6 each from a stalk. Rind hardness measured at II and IV for the middle internode was omitted to avoid preponderance of this internode, thus creating a bias in its favour.

TABLE IV

Estimated variance due to different zones in the rind hardness in the middle portion of a stalk for three sugarcane varieties (1950–51)

Varieties	Mean	S.E.	S.E. as % of the mean	σ_1^2	σ_1^2 expressed as % of total variance	σ_2^2	σ_2^2 expressed as % of total variance	σ_3^2	σ_3^2 expressed as % of total variance	σ_4^2	σ_4^2 expressed as % of total variance
Co. 453	12·06	0·5352	4·44	0·0151	0·31	0·7848	16·37	0·1313	2·74	3·8639	80·58
Co. 513	11·78	0·1509	1·28	0·0397	2·10	0·1196	6·32	0·1027	5·42	1·6316	86·16
B.O. 11	10·89	0·1557	1·43	0·0000	0·00	0·3196	11·17	0·0933	3·26	2·4480	85·57

1. σ_1^2 = Variance due to rows. σ_2^2 = Variance due to units. σ_3^2 = Variance due to clumps. σ_4^2 = Variance due to stalks.

2. If the mean square due to a particular hierarchy happens to be less than that in the immediately next one, the variation due to that hierarchy has been taken to be zero.

variance due to this zone was nil in seven out of nine varieties investigated by them, it being 3·44% and 1·44% of the total variance respectively in Co. 313 and Saharanpur Paunda (*loc. cit.*, Table III). Moreover averages for this character in different sectors, when considered separately or in their various combinations, did not differ significantly from the average for the variety, nor did the small differences between them show any consistent trend of variation in favour of any one sector or a combination (*loc. cit.*, Table IV). Although in the case of the lignification of cell-walls, both in parenchymatous and sclerenchymatous tissues and of the cell-size in the former, sectors were, however, found to be responsible for a fairly large amount of variation in the sample, they did not effectively reflect themselves in the magnitude of rind hardness and thus the predominant position assigned, by Khanna and Panje (1939) and Khanna and Sharma (1947) to the vascular area amongst the anatomical characters, on theoretical grounds, has been amply justified by the present investigations.

Internode as a hierarchy was found to be very important because it contributed approximately as much as 80% or more to the total variance in the sample, and hence needed all the care in its selection. It might, however, be stated that although rind-like juice quality or fibre content varied from internode to internode, differences were of such a low order as to be negligible. Ueno (1938) found that the rind hardness not only of internode (proper) but also of bloom-band and root zone of lowest internode of middle region was more or less the same as that of the uppermost internode of the bottom region. An internode just below and above the middle could, therefore, be taken if the latter happened to be unfit for observation.

In the case of stalk which was common to both the methods of sampling, its importance was determined to a great extent by the method itself. In 1949–50 sample, its contribution to the total variance in the sample was practically nil while in 1950–51 it was as high as 86·16%. Of the remaining three hierarchies, unit as already stated was able to bring in, by itself, much more variation than clump or row or both put together.

It is interesting to note that values for Co. 453 and B.O. 11 obtained by method B were 91·64% and 89·40% respectively, of those arrived at by method A (Table V). Although in both the cases they were in the neighbourhood of 90%, it cannot be stated definitely that this ratio would hold good for all varieties because data were too meagre for such a generalization.

B. Sample size.—In view of the considerations mentioned above, the size of sample will depend upon the method that is to be employed. If

TABLE V

Average hardness and estimated size of sample for varieties studied in 1949–50 *and* 1950–51 *within a margin of error of* 5%, 2½% *and* 1% *of the mean*

(*a*) Method A

Varieties	Average hardness	At 5%			At 2½%			At 1%		
		Canes	Internodes	Sectors	Canes	Internodes	Sectors	Canes	Internodes	Sectors
Co. 453	.. 13·16	13	1	1	51	1	1	317	1	1
B.O. 10	.. 12·06	18	1	1	72	1	1	454	1	1
B.O. 11	.. 12·18	11	1	1	42	1	1	264	1	1
B.O. 21	.. 10·01	25	1	1	98	1	1	616	1	1
B.O. 24	.. 12.18	16	1	1	65	1	1	407	1	1

TABLE V

(*b*) Method B

Varieties	Average hardness	At 5%					At 2½%					At 1%				
		Rows	Units	Clumps	Canes	Observations to be taken	Rows	Units	Clumps	Canes	Observations to be taken	Rows	Units	C ump	Canes	Observations to be taken
Co. 453 ..	12·06	1	20	1	2	40	1	113	1	2	226	8	78	1	2	1348
		2	10	1	2	40	2	44	1	2	176	10	58	1	2	1160
		3	7	1	2	42	3	27	1	2	162	12	46	1	2	1104
Co. 513 ..	11·78	1	10	1	2	20	2	28	1	2	112	8	108	1	2	1728
		2	40	1	2	16	3	18	1	2	108	84	48	1	2	960
		3	3	1	2	18	4	10	1	2	80	12	31	1	2	744
B.O. 11 ..	10·89	1	13	1	2	26	1	50	1	2	100	8	39	1	2	625
		2	7	1	2	28	2	25	1	2	100	10	31	1	2	620
		3	5	1	2	30	3	17	1	2	102	12	26	1	2	634

the amount of material to be tested happens to be small in quantity, as is the case with sugarcane varieties in the early stages of multiplication, method used in 1949–50 would be quite useful, while varieties covering larger area should better to tested with that of 1950–51. The former no doubt is quick and can, at best, be used for comparative purposes only. It is, however, not as accurate as the latter, when overall picture of a variety is sought.

From the estimated sample size, given for the six varieties, it would appear tha talthough it was different for different varieties, 25, 100 and 500 canes would give the average rind hardness, within a margin of 5%, 2½% and 1% error of the mean, in most of the varieties by method A. For the method B, it would be respectively 50, 250 and 1,250 observations taken from the four hierarchies as shown in Table V. If on the analysis of data, so collected for a variety it is found that the margin of error happens to be greater than that allowed for, the size of the sample will have to be enlarged in the direction indicated by analysis with a view to see exactly which of hierarchies is contributing more than its normal share towards the total variation in the sample.

All measurements should be taken on the side opposite the bud at the mid-point of the middle internode of stalks.

V. Summary

1. Rind hardness was measured for five varieties from 100 selected stalks in 1949–50 and for three varieties from all millable cane within 1/40th of an acre in 1950–51.

2. (*a*) Sector, clump, unit and row as hierarchies were not found to be as important as internode or stalk.

(*b*) Unit brought in more variation within the sample than the clumps.

3. The size of sample by the first method would be 25, 100 and 500 well-developed stalks, the best of a clump, to estimate this character within margin of 5%, 2½% and 1% error of the mean. For the second method, the number of observations required would be 50, 250 and 1,250 taken from the four hierarchies according to a certain plan.

4. All measurements should be done on the side opposite the bud at the mid-point of middle internodes.

VI. ACKNOWLEDGEMENTS

These investigations were carried out at the Central Sugarcane Research Station jointly financed by the Bihar Government and the Indian Central Sugarcane Committee to whom grateful thanks are due. The authors are indebted to Shree K. L. Khanna, Director, Sugarcane Research and Development, for placing necessary facilities at their disposal and for helpful criticism during the course of the work. Assistance rendered by Shree J. P. Pandey in the analysis of the data presented in this paper and by the Botany Staff in their collection in 1950–51 season, is also thankfully acknowledged.

VII. REFERENCES

1. Buzacott, J. H. .. "The relationship between hardness of sugarcane and varietal resistance to beetle borer (*Rhabdocnemis obscura* Boisd)," *Tech. Commun. Bur. Sug. Ext. Sta., Queensland*, 1940, **8**, 127–52.

2. Khanna, K. L. .. "An improved instrument for testing rind hardness in sugarcane," *Agr. and Livestock in India*, 1935, **5**, 156–58.

3. —— and Panje, R. R. .. "Studies in the rind hardness of sugarcane. I. Anatomy of the stalk and rind hardness," *Ind. Jour. Agri. Sci.*, 1939, **9**, 1–14.

4. —— and Sharma, S. L. .. "Studies in the anatomy of sugarcane stalk—I. Chewing canes," *Proc. Ind. Acad. Sci.*, 1947, **26**, 13–31.

5. ———, ——— .. "Studies in the anatomy of sugarcane stalk—II. Milling characteristics of varieties," *Ibid.*, 1949, **29**, 23–34.

6. ———, ———, and Bandyopadhyay, K. S. "Studies in anatomy of sugarcane stalk—IV. Sampling technique for the evaluation of anatomical structure for comparative study," *Ibid.*, 1950, **31**, 1–33.

7. Rao, J. Thuljaram .. "Rind hardness as a possible factor in resistance of sugarcane varieties to stem borer," *Curr. Sci.*, 1941, **10**, 365–66.

8. ——— .. "Anatomical character of stem in relation to rind hardness and borer attack in sugarcane," *Proc. First Bienn. Conf. of Sugarcane Research Workers in the Indian Union, Coimbatore*, 1951, 41–47.

9. Ueno, T. .. "Relation of rind hardness to the internal structure of sugarcane," *Rept. Govt. Sug. Exp. Sta. Tainan. Formosa*, 1938, **5**, 21–22.

1078–57. Printed at The Bangalore Press, Bangalore City, by C. Vasudeva Rao, Superintendent, and Published by The Indian Academy of Sciences, Bangalore.

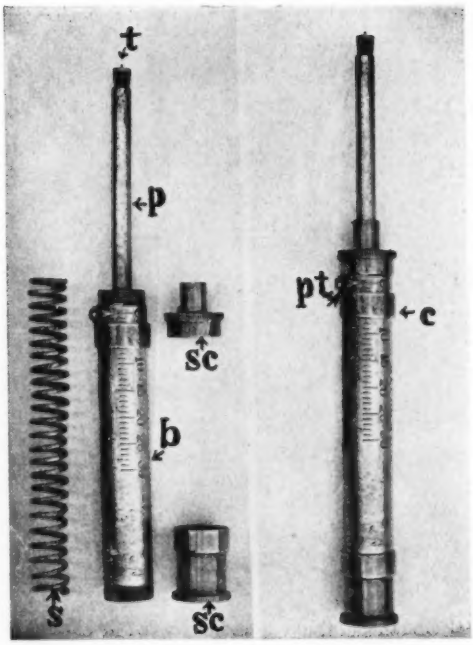


FIG. 1. Rind hardness apparatus and its components, namely the spring (*s*), the graduated barrel (*b*), with indicator-collar (*c*), plunger (*p*) with indicator (*pt*) at the base and puncturing point (*t*) at the top, and two screw-caps (*sc*). (*After Khanna.*)

Sup

Acha
Balak
Bal, I
Balar
Bilgra
Desik
Desik
Bal
Devi,
Jayar
Ses
Khan
L.
LaFo
Laksh
Ver
Mara
D.
Miras

Nadk
Sre

Naray
B. F
M.
Natara

Nigam

Supplement to the

"Proceedings of the Indian Academy of Sciences"

INDEX TO VOL. XLVI—(B)

AUTHORS' INDEX

Acharya, A. K.	..	*See* Singh and Acharya.
Balakrishnan, M. S.	..	*See* Desikachary and Balakrishnan.
Bal, D. V.	..	*See* Marathe and Bal.
Balaramamurty, C.	..	*See* Ramasastry and Balaramamurty.
Bilgrami, K. S.	..	*See* Tandon and Bilgrami.
Desikachary, T. V.	..	Electron microscopy and algology, 54.
Desikachary, T. V. and Balakrishnan, M. S.		*Tænioma* J. Ag. from India, 336.
Devi, Maheswari	..	Embryological studies in Compositæ, III, 68.
Jayaraman, R. and Seshappa, G.		Phosphorus cycle in the sea with particular reference to tropical inshore waters, 110.
Khanna, K. L., Nigam, L. N. and Puri, V. D.		*Chilo tumidicostalis* Hampson—a serious stem borer pest of sugarcane in Bihar, 75.
LaFond, E. C.	..	Oceanographic studies in the Bay of Bengal, 1.
Lakshmanan, M. and Venkata Ram, C. S.		Influence of *fusarium* culture filtrates on respiratory changes in cotton, 131.
Marathe, V. B. and Bal, D. V.		The development of the Chondrocranium in *Trichopodus trichopterus* (Pall.), 347.
Mirashi, M. V.	..	Contribution to our knowledge of the physiological anatomy of some Indian hydrophytes, VI, 247.
Nadkarni, G. B. and Sreenivasan, A.		The metabolism of serine in pyridoxine deficiency, 138.
	..	The metabolism of serine in biotin deficiency, 229.
	..	The metabolism of serine in folic acid deficiency, 236.
Narayanan, E. S., Rao, B. R. Subba and Rao, M. Ramachandra		*Hymenia recurvalis* F. and its parasite complex, 241.
Natarajan, A. V.	..	Studies on the egg masses and larval development of some prosobranchs from the Gulf of Mannar and the Palk Bay, 170.
Nigam, L. N.	..	*See* Khanna and others.

Padmanabhan, S. Y.	..	The relation between loss in viability and seed-borne micro-flora in rice, 155.
Prasad, Rajeshwar	..	*See* Sharma and Prasad.
Puri, V. D.	..	*See* Khanna and others.
	..	Studies on the anatomy of the sugarcane moth borers, III, 96.
Ramakrishnan, T. S.	..	Notes on some fungi from South India—VI, 149.
Ramasastry, A. A. and Balaramamurty, C.		Thermal field and oceanic circulation along the East Coast of India, 293.
Rao, B. R. Subba	..	*See* Narayanan and others.
	..	Some new species of Indian hymenoptera, 376.
Rao, M. Ramachandra	..	*See* Narayanan and others.
Rao, N. S. Subba	..	Translocation of griseofulvin in rice seedlings (*Oryza sativa* L.), 65.
Seshappa, G.	..	*See* Jayaraman and Seshappa.
Sharma, S. L.	..	A new report of a rust [*Puccinia kuehnii* (Krueg) Butler] on *Erianthus munja*, 126.
Sharma, S. L. and Prasad, Rajeshwar		Studies in sampling technique, VI, 391.
Singh, Inderjit and Acharya, A. K.		Ionic changes in unstriated muscle immersed in sodium-free solutions, 47.
	..	Excitation, inhibition, contraction, action potentials and conduction in unstriated muscle in the absence of any electrolyte in the external medium, 285.
Sreenivasan, A.	..	*See* Nadkarni and Sreenivasan.
Subramanian, C. V.	..	Hyphomycetes, IV, 324.
Tampi, P. R. S.	..	Some observations on the reproduction of the milk-fish *Chanos chanos* (Forskål), 254.
Tandon, R. N. and Bilgrami, K. S.		Assimilation of disaccharides by some fungi causing "Leaf spot" diseases, 274.
Venkata Ram, C. S.	..	*See* Lakshmanan and Venkata Ram.

TITLE INDEX

Chilo tumidicostalis Hampson—a serious stem borer pest of sugarcane in Bihar (Khanna and others), 75.

Compositæ, embryological studies, III (Devi), 68.

Disaccharides, assimilation, by some fungi causing "leaf spot" diseases (Tandon and Bilgrami), 274.

Egg masses and larval development of some prosobranchs from the Gulf of Mannar and the Palk Bay, studies (Natarajan), 170.

Electron microscopy and algology (Desikachary), 54.

Fungi from South India, some, notes, VI (Ramakrishnan), 149.

Fusarium culture filtrates, influence, on respiratory changes in cotton (Lakshmanan and Venkata Ram), 131.

Griseofulvin in rice seedlings (*Oryza sativa* L.), translocation (Rao), 65.

Hydrophytes, some Indian, physiological anatomy, contribution to our knowledge (Mirashi), 247.

Hymenia recurvalis F. and its parasite complex (Narayanan and others), 241.

Hymenoptera, Indian, some new species (Rao), 376.

Hyphomycetes, IV (Subramanian), 324.

Milkfish *Chanos chanos* (Forskål), reproduction, some observations (Tampi), 254.

Muscle, unstriated, excitation, inhibition, contraction, action potentials and conduction in, in the absence of any eletcrolyte in the external medium (Singh and Acharya), 285.

Muscle, unstriated, immersed in sodium-free solutions, ionic changes (Singh and Acharya), 47.

Oceanographic studies in the Bay of Bengal (LaFond), 1.

Phosphorus cycle in the sea with particular reference to tropical inshore waters (Jayaraman and Seshappa), 110.

Rice, loss in viability and seed-borne micro-flora, the relation between (Padmanabhan), 155.

Rust, [*Puccinia kuehnii* (Krueg) Butler] on *Erianthus munja*, a new report (Sharma), 126.

Sampling technique, studies, VI (Sharma and Prasad), 391.

Serine, the metabolism, in biotin deficiency (Nadkarni and Sreenivasan), 229.

Serine, the metabolism, in folic acid deficiency (Nadkarni and Sreenivasan), 236.

Serine, the metabolism, in pyridoxine deficiency (Nadkarni and Sreenivasan), 138.

Sugarcane moth borers, studies on the anatomy, III (Puri), 96.

Tænioma J. Ag. from India (Desikachary and Balakrishnan), 336.

Thermal field and oceanic circulation along the East Coast of India (Ramasastry and Balaramamurty), 293.

Trichopodus trichopterus (Pall.), Chondrocranium in, the development (Marathe and Bal), 347.